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CONTROL OF EXTREMITY SKIN TEMPERATURE IN COLD ENVIRONMENTS - THE ROLE OF MAST CELLS, HISTAMINE AND THE SYMPATHETIC NERVOUS SYSTEM

by

© Gordon Arthur Hills

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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IN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Control of Extremity Skin Temperature in Cold Environments - The Role of Mast Cells, Histamine and the Sympathetic Nervous System submitted by Gordon Arthur Hills in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Physiology

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ABSTRACT

Three series of experiments were conducted to determine the role of mast cells, histamine and the sympathetic nervous system in the response of peripheral tissues to acute cold in cattle and sheep.

In the first series of experiments with steers and sheep mast cells were found to be distributed throughout the dermis, particularly in association with blood vessels and the connective tissue surrounding sebaceous glands and hair follicles. Mast cells were more abundant in the superficial than the deeper layers of the dermis but were not found in the epidermis. The mean number of mast cells/mm^2 of dermal cross section of the steers ranged from 729 to 2317 in midside skin samples, from 1024 to 1354 in skin from the upper foreleg and from 1403 to 2672 in ear skin in the steers. There was no significant effect of environmental temperature or feed intake level on the number of mast cells in any of the sample sites.

In the second series of experiments carotid artery infusions of compound 48/80 (0.1 to 1 ml/kg) in sheep did not have any consistent effect on ear skin temperature of cold (-27C) exposed sheep, but did result in pronounced increases in heart rate. Subcutaneous injections of 500 µg of histamine - HCl directly into the ear, resulted in an

increase in ear surface temperature from 5.6 to 26.8C. Prior treatment with the H_1 receptor blocking agent, benadryl, (0.2 to 0.375 mg/kg) reduced the dilatation effect of histamine but did not alter the size or frequency of the "hunting response" in cold exposed sheep. Higher doses of benadryl (0.5 to 0.75 mg/kg) did not inhibit the dilatory effect of histamine. The results suggest that there may be high affinity H_1 dilatory receptors low affinity H_1 constrictor receptors and H_2 dilatory receptors influencing the vasculature in the ears of sheep.

During the third series of experiments temperatures recorded from six sites on individual ears showed synchronous fluctuations during each "hunting response". Intravenous (2.0 µg/kg) or direct subcutaneous injections of isoproteranol (100-400 µg) did not influence the ear skin temperature or alter the "hunting response". During exposure of sheep to 20C, the resting plasma concentrations of adrenaline and noradrenaline were 0.05 ng/ml and 0.46 ng/ml respectively for the jugular vein and 0.05 ng/ml and 0.33 ng/ml for the carotid artery samples. After 90 min exposure to -20C, plasma concentrations of adrenaline and noradrenaline were 0.10 ng/ml and 0.80 ng/ml respectively for the jugular vein and 0.12 ng/ml and 0.66 ng/ml respectively for the carotid artery samples. Concentrations of adrenaline and noradrenaline in jugular plasma from the

intact and sympathectomized sides of the head were 0.22 ng/ml and 0.16 ng/ml for adrenaline and 0.98 ng/ml and 0.53 ng/ml for noradrenaline during exposure to -15.8C. Mean jugular vein plasma concentrations of adrenaline and noradrenaline were 0.11 ng/ml and 0.56 ng/ml during the dilating phase and 0.17 ng/ml and 0.65 ng/ml during the constricting phase of individual "hunting responses". Both noradrenaline and adrenaline proved to be significantly greater ($P<0.01$ and 0.05) during the constriction phase of individual "hunting responses".

Severing the cervical sympathetic nerve trunk to a cold exposed ear in anaesthetized sheep resulted in an immediate increase in ear surface temperature. Electrical stimulation of the nerve caused a decrease in ear temperature to a constant value near freezing. There were large auricular arterio-venous differences in noradrenaline only at times when the ear temperature was decreasing. The results suggest that the "hunting response" in the ears of sheep is due to a temporary interruption in activity of sympathetic adrenergic neurons to the larger blood vessels of the ear. This withdrawl of sympathetic stimulus may be due to a somatosympathetic reflex which causes a withdrawl of sympathetic stimulation to an individual extremity if the cold exposure is noxious enough.

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DEDICATION

This thesis is dedicated to my wife Cicely Joan Hills, whose courtship and early marriage suffered as only she will ever know, that this work might be accomplished.

INTRODUCTION

When faced with an acute cold challenge homeothermic animals have a limited number of responses available to maintain the consistency of their internal temperature. The internal temperature of "warm blooded" animals was considered by Cannon (1939), to be "One of the most striking and easily observed constants of the internal environment..". In order to maintain this relatively constant internal temperature during acute cold exposure mammals make use of behavioral and physiological adjustments. The latter includes peripheral vasoconstriction in order to reduce heat loss to the environment. The advantage of reducing peripheral blood flow and therefore reducing surface temperature can be seen by examining Fournier's Law of heat flow as presented by Kleiber (1975):

$$q = \lambda \frac{A}{L} (Tb-Ts)$$

where:

q = rate of heat flow from an animal per unit time

L = thermoconductive thickness of the insulating layer

λ = heat conductivity of insulating layer

Tb= temperature of the core

Ts= temperature of the skin

A = surface area

It can be readily seen that, in order to maintain Tb constant in a condition where Ts is declining, any factor such as a reduction in skin blood flow, which serves to decrease λ or to increase L will reduce the magnitude of q.

Webster (1974a) notes that in the extremities (which he defines as "those parts of the head and limbs which have little muscle or visceral tissue capable of producing heat in situ"), alteration of blood flow is the only effective mechanism available to modify heat exchange. He further suggests that "the study of temperature regulation in the extremities is, therefore, the study of blood flow to these regions". Whittow (1962) using Brody's (1945) formula and measurement of the surface area of the skin estimated that the extremities of cattle constituted 30 percent of the surface area of the animal. Thus control of the peripheral circulation is one of the major mechanism of controlling heat loss from an animal.

The response of the cardiovascular system to a cold environment can be divided into two major responses. First, there are increases in blood flow to tissues with high metabolic activity such as brown adipose tissue and muscle as measured by Alexander et al. (1963) in lambs and by Jansky and Hart (1968) in rats. Second, there is a reduction in blood flow to peripheral tissues and extremities resulting in a functional increase in tissue insulation as discussed by Webster (1974a and b).

Although it is of value for an animal to be able to reduce blood flow to its superficial tissues and particularly to its extremities to prevent heat loss (Webster, 1974a), there is a finite limit to the reduction which can take place if the animal is to prevent its extremities from freezing at sub-zero temperatures. For example, Wilson et al. (1976) have shown that finger skin will freeze at a mean surface temperature of -9.4C.

A phenomenon which may help to prevent tissue damage due to freezing is the occurrence of periodic increases in the surface temperature, the "hunting phenomenon" as it was called by Lewis (1930) who first described the reaction in cold exposed fingers. This phenomenon has subsequently been extensively studied. However, no satisfactory explanation of the mechanism of the periodic rapid increases in blood flow resulting in rapid increases in surface temperature of the extremities has been forthcoming.

The constriction of peripheral blood vessels in response to cold has been attributed to the vasoconstrictor effect of noradrenaline released from peripheral sympathetic nerve fibers. It has been shown (Meyer and Webster 1971) that the surgically sympathectomized ear of a sheep, failed to vasoconstrict when the animal was housed in a cold environment of -17C. However, if noradrenaline was infused into the animal during cold exposure, the ear temperature decreased as a result of vasoconstriction. Hope *et al.* (1976) also reported a constriction in the isolated rabbit ear artery in response to stimulation of the adrenergic sympathetic nerve supplying the vessel.

Several authors have noted a lack of synchrony of the "hunting response" in different extremities. Lewis (1930) reported that the fingers of a cooled hand "hunted" independently of each other. Greenfield *et al.* (1951) reported that the commencement of cold induced vasodilatation in an extremity was independent of the temperature of the other extremity and depended only on the length of time that the hand cooled. Meyer and Webster (1971) reported an asynchronous nature of the fluctuations in skin temperature of different extremities in the sheep. This independent action of different extremities of the body lead Meyer and Webster (1971) to suggest a local control of cold induced vasodilatation. Meyer (1970) divided the possible local mechanisms accounting for vasodilatation during the "hunting response" into five categories: (1) an increase in vasodilatory substance or substances (2) the liberation of a noradrenaline inhibitor (3) a shut off of noradrenaline (4) a decrease in tissue sensitivity to noradrenaline or (5) any combination of (1), (2), (3), (4).

Numerous naturally occuring constituents of the body are known to have vasodilatory properties either through their direct action on vascular smooth muscle or through their ability to prevent the constriction activity of sympathetic nerves. Many of these constituents have been proposed as the causative agent of vasodilation under various circumstances. Hurley and Mescon (1956) and Nelms (1963) reported a direct cholinergic innervation of arterio-venous anastomoses while Duff et al. (1953) had previously reported normal cold induced vasodilation in a fully atropinized finger. This latter observation would seem to rule out the likelihood of an involvement of a cholinergic dilatory mechanism. Bradykinin causes dilatation of human veins and resistance vessels (arterioles). Kontos et al. (1964) found bradykinin infusion into the forearm (25 µg/min) increased blood flow to the hand while 40 µg/min was required to increase blood flow in the forearm. Mason and Melman (1965) reported a constriction of short duration followed by a more sustained dilatation in the forearm in response to sudden injections of 20 to 35 µg bradykinin. They concluded that the direct effect of the drug on the systemic smooth muscle of both forearm arterial and vascular beds were similar. Collier *et al.* (1972b) reported that 4-128 ng/ml bradykinin produced dilatation in dorsal hand veins which had either been constricted with noradrenaline or 5-hydroxytryptamine. During the initial stages of cold exposure (0C) of the hand Cuschierri *et al.* (1970) found the venous kininogen level rose, indicating to them that in the initial constriction of the blood vessels there was a decrease in the normal kininogen utilization at the capillary level in the fingers. Plasma concentration of kininogen decreased during the dilatation phase of the immersion of the hand in cold water indicating a release of kinin in this phase. They considered this to be evidence supporting Armstrong's (1965) hypothesis of cold activation of the kininogenase precursor. That is, during the cold constricted phase the lowering of the plasma temperature leads to an increase in concentration of the active kininogenase and hence the generation of kinins such as bradykinin. The increased kinins, being vasodilators, cause an opening of the arterio-venous anastomases, increasing the blood flow, washing away the kinin and allowing the entire process to repeat itself. However, these authors did not consider the possibility that a consistent outflow of kininogenase may have been diluted by the increased blood flow to the tissue. Mashford and Zacest (1967) found, however, a decline in the output of bradykinin in the venous flow from the hand during immersion in cold water, a result which they tock to indicate that bradykinin was not a mediator of this response. Also, in the rat, which displays cold induced vasodilatation in the tail (Helstrom 1975), and in the rabbit, in which the ears display the "hunting response" as shown by Harada and Kanno (1975), bradykinin appears to be a vasoconstrictor in the peripheral vasculature. Rowley (1964) found that injection of 0.02 ml of a 40 µg/ml solution of bradykinin adjacent to a cutaneous vein and artery in rats caused a constriction of the vein within 5 to 15 s with a return to normal diameter in 5 to 6 min. A similar partial constriction of the artery was noted. Bobbin and Guth (1968) found by direct observation of the marginal ear artery in the rabbit that 0.08 to 20 ng bradykinin caused a reduction of about 50 percent in the vessel diameter in both intact and denervated anesthetized rabbits. Thus the possibility that bradykinin is the active local dilator in the "hunting response" is still in doubt.

Prostaglandins (PG) may fit two of Meyer's (1970) categories in that PGE_1 and PGE_2 have been shown by Hedquist (1969, 1970) to decrease the overflow of ^3H-noradrenaline from the feline spleen after nerve stimulation. This led him to the hypothesis that PGE_2 locally mobilized by sympathetic nerve stimulation may counteract further release of noradrenaline by a negative feedback mechanism thus exerting a braking effect on the neuroeffector system. Hadhazy *et al.* (1976) found that PGE_1 produced a dose dependent inhibition of the constrictor responses to nerve stimulation of the isolated perfused rabbit ear artery. Prostaglandin E_2 did not affect the response to noradrenaline and it markedly reduced the stimulation induced release of ^3H-noradrenaline from arteries incubated in ^3H-noradrenaline. They concluded from these studies that the action of PGE_1 was to prevent the release of noradrenaline from its storage sites. Kadowitz *et al.* (1971a and b) found that PGE_2 reduced the vasoconstrictor responses in the hind limb of the dog to intraarterial infusions of noradrenaline as well as to nerve stimulation. Greenberg and Sparks (1969) found that PGE_1 and PGA_1 caused relaxation of isolated venous smooth muscle taken from the hind leg of the dog. Hedwall *et al.* (1970) found that PGE_1 did not cause dilatation in the isolated hind paw of the dog but did prevent constriction due to nerve stimulation or to noradrenaline infusion. They concluded that the inhibition of constriction caused by PGE_1 was not associated with nor directly dependent on the direct dilator action of this PG. Daugherty (1971) infused PGE_1 into the isolated skin and muscle vasculature of the dog's forelimb with an intact nervous system. He suggested that PGE_1 produced an active vasodilatation as in his experiments under a constant flow PGE_1 caused a dilatation of the blood vessels which could not be attributed to passive distention of the vessels due to an increase in flow. However, as the nerves were intact it could not be determined whether this response was a withdrawl of sympathetic constrictor activity or a direct response on the smooth muscle. During an examination of the effects of PG on the uterine circulation of the dog, Clark _et al._ (1970) demonstrated that intra arterial infusions of low rates of PGE_1 (100 ng/min) reduced the vasoconstricting response to noradrenaline administered at 0.1 and 1 µg/min. (vasoconstriction) and to low frequency stimulation (3Hz). As both response to noradrenaline and stimulation were depressed, Clark _et al._ (1970) suggest that in the canine uterus PGE_1 has a post junctional effect on the response to sympathetic stimulation of the vasculature. Kadowitz _et al._ (1971a, 1972) suggest that in the hind paw of the dog the action of PGE_2 is prejunctional and serves to fascilitate the release of noradrenaline while in the hindlimb PGE_2 significantly reduced responses to both intraarterial noradrenaline and to nervous stimulation. Kadowitz (1972) concluded that PGE_2 caused a postjunctional depression of the response to catecholamines. However in spite of the fact that PG appear to be able to affect the constriction of blood vessels in response to sympathetic stimuli at both prejunctional and postjunctional sites there is at present no evidence to indicate whether PG's are involved in the "hunting response" of cold exposed extremities.

The intermediates of the Krebs TCA cycle have also been suggested as possible vasodilatory agents by Frohlich (1965). However, Haddy and Scott (1968) felt that the vasodilator acton of each individual component was weak, and only if these effects were additive would a measureable dilatory response be seen.

Accumulation of catecholamines within a tissue might have a negative feedback effect temporarily reducing the release of noradrenaline from the nerve endings. Hope et al. (1976) found that infused noradrenaline (0.5 and 5 µm) inhibited transmitter release in isolated rabbit ear arteries in which the noradrenergic transmitter stores were labelled with ^{3}H-noradrenaline. Sanders and Ross (1975), found that intraarterial infusions of noradrenaline and dopamine markedly attenuated the mesenteric vasoconstrictor response to periarterial nerve stimulation in anesthetized cats. Steinsland and Hieble (1978) reported that dopamine injected at concentrations ranging from 3 to 1000 nM produced a concentration dependent inhibition of the constrictor responses of the isolated perfused rabbit ear artery to brief periods of nerve stimulation.

Iriki and Hales (1976) suggested that increases in skin temperature of anesthetized rabbits may be due to temporary decreases in activity of the sympathetic vasoconstrictor nerve. They observed spontaneous oscillations in ear skin temperature which were accompanied by "inverse changes of up to 90 percent in the electrical activity of the postganglionic nerve twig accompanying one of the retroauricular arteries" in anesthetized rabbits. Whether this mechanism is involved in the "hunting response" remains to be determined.

A number of authors have reported a change in vascular constriction with lowered tissue temperature. Nagasaki and Carlson (1971) found that decreasing the temperature of the blood from 40 to 20C in a perfused rabbit ear with the auricular nerves intact caused an increase in vascular resistance. Further cooling to 10C decreased vascular resistance in warm acclimated (25C) rabbits. They suggested that the decreased vascular resistance with lower blood temperature may have been due to the "cold block of sympathetic nerves" as reported by Reite _et al._ (1966) or to decreased sensitivity of the vascular smooth muscle to noradrenaline in the cold. Millard and Reite (1975) however, using the isolated perfused leg of the domestic duck (_Anas boscas_) have shown that peripheral veins will constrict in response to noradrenaline at temperatures as low as 2C. However, the dose required to cause the constriction was quite large (1-5 µg).

Histamine has long been considered a possible regulator of the circulatory system as shown in the early reports of Bayliss (1902) and Lewis (1927). More recently revived interest in histamine as a neuromediator came about when it was revealed that certain peripheral vasodilatations could be blocked by antihistamines. Tuttle (1965) found that the hypotensive response evoked by stimulation of a narrow zone dorsal to the median raphe of the posterior hypothalamus was accompanied by an increase in blood histamine concentration. This dilatory response could be prevented by chlorpheniramine maleate an antihistamine. Atropine, which caused a similar systemic dilatation, was also not effective after the antihistamine. They suggested that acetycholine might be responsible for the liberation of histamine which then produced the dilatation. In the isolated but denervated hindquarters of the dog, Beck (1965) found that the antihistamine triplenamine reduced the reflex dilatation induced by intravenous injection of adrenaline and noradrenaline. He proposed that the active component of the reflex vasodilatation was mediated by neurologically released histamine. Although Brody (1966) was unable to detect an increase in histamine outflow into the blood of dogs during reflex dilatation he was able to demonstrate an increased outflow of previously taken up ^{14}C-histamine and its breakdown product methyl-^{14}C-histamine during the reflex dilatation caused by infusion of adrenaline and noradrenaline. Blood concentrations of methyl-^{14}C-histamine were also found to significantly increase in the active dilatation phase following section of the lumbar sympathetic chain. These results are also suggestive of the participation of histamine in the active portion of reflex vasodilatation.

Histamine responses are considered to be mediated by at least two receptor sites (H_1, H_2) the first of which was defined as H_1 by Ash and Schild (1966). The production of H_2 receptor inhibitors (Black et al. 1972) added further stimulus to the investigation of a possible role for histamine in the control of the vascular system. Parsons and Owen (1973) utilized burimamide, the first of the H_2 blockers to be developed, to demonstrate both H_1 and H_2 receptors for histamine in the vasculature of the anesthetized dog, cat, and rabbit. In the isolated ear of the rabbit they found that activation of H_1 receptors appeared to cause a constriction while activation of H_2 receptors appeared to cause a dilatation when activated by exogenous histamine. The presence of both types of receptors was also reported in the vasculature of the dog by Powell and Brody (1973). In addition, Powell and Brody (1973) found that the dilatation of the perfused gracilis muscle of the dog could be reduced below that caused by mepyramine, an H_1 blocker, when burimamide was also given. Lorenz *et al.* (1973) found that the H_1 blocking agent dimethindene maleate and the H_2 blocking agent metiamide diminished the hypotensive response to the histamine released from mast cells by Chemophor El in spite of an elevation of plasma histamine in response to Chemophor El before and after the metiamide. Glover *et al.* (1973) found that the constrictor response to histamine by the isolated rabbit ear artery became a dilator response when H_1 receptors were blocked by mepyramine. This dilator response may be blocked by burimamide or metiamide. These authors therefore suggested that these blood vessels could contain both H_1 and H_2 receptors having opposite responses to histamine. On the other hand, human temporal arteries showed no increase in constriction in response to histamine. However histamine produced dose dependent reductions in the constrictor response to noradrenaline. This action of histamine was unaffected by mepyramine but was antagonized by burimamide and metiamide suggesting to these authors that human temporal arteries contained only H_2 receptors. Thus evidence exists for the participation of histamine in modulation of the vascular response to noradrenaline as well as for a direct effect of histamine on the vessels.

The source of the histamine acting on the blood vessels is still a matter of debate. However, the above results of Lorenz *et al.* (1973) show that histamine released from mast cells is capable of reacting with receptors in the vascular system. Selye (1965) has presented anatomical evidence for a close association between mast cells and blood vessels. Mast cell numbers have also been shown by LeBlanc and Rosenberg (1957a) to change in rats in response to a cold environment. These observations suggest the mast cells, through histamine release, might play a role in the "hunting response". Thus a number of agents in the animal body could be responsible for the dilatation of the sympathetically constricted blood vessels of the cold exposed periphery that is "hunting".

Blood flow to the periphery during acute cold exposure is related to the functioning and possibly to the survival of human beings. Livingston (1974) suggested that an increased constrictor tone of the fingers of military personnel during short term (2 week) cold exposures on arctic exercises might affect their comfort and dexterity. In agreement with this suggestion Bensel and Lockhart (1974) reported that the early onset of cold induced vasodilatation in the hands appeared to be associated with an initially superior performance and a subsequent inferior performance of specific manual tasks with increased duration of whole body cooling. The control of peripheral circulation may also be related to the overall survival of animals or humans when faced with a sudden cold exposure. Greenfield et al. (1951b) were able to demonstrate that emersing the hands alone in ice water would cause a decline in the oesophageal temperature from 36.5 to 35.95C in as few as 8 min. The temperature did recover slightly to 36.1C but remained low for the duration of the trial.

Therefore, the importance of the control of peripheral circulation to human and animal function and survival in the cold has lead to continued research in the area. Helstrom (1975) reported cold induced vasodilatation in the tails of rats housed at 23C and tested in a chamber at 36C when the tails were exposed to cold water. Reite et al. (1977) concluded from studies on the feet of domestic ducks that the failure of the tissues to responsd to adrenaline and noradrenaline in the cold was the cause of cold induced vasodilatation. They reported, however, that they were able to produce constriction in the cold (4C) perfused foot with infused noradrenaline (0.1 µg).

Thus although many vasodilatory mechanisms occur in the body, some of which have been shown to be potentially involved in the hunting response, no totally satisfactory explanation of the dilatory phase of the "hunting response" has been presented. Therefore the present study was undertaken to further investigate the "hunting response".

The three major aspects of this study were to investigate (a) the distribution of mast cells as a possible source of histamine in cattle and sheep skin, (b) the potential role of histamine as a dilating agent in the skin of sheep and, (c) the role of the sympathetic nervous system in the control of vasoconstriction and vasodilatation in the peripheral skin of sheep.

Chapter 1 - Mast Cells in the Skin of Warm and Cold Exposed Cattle

LITERATURE REVIEW

Selye (1965) defines a mast cell as a "connective tissue element which possesses cytoplasmic granules that stain metachromatically under ordinary conditions". These cells were first characterized by Ehrlich in 1879 (Selye 1965) who recognized the tendency of the cells to be located around blood vessels, nerves, and glandular ducts as well as in inflamatory and neoplastic foci.

Prolonged cold exposure in man (LeBlanc _et al._ 1960), in hedgehogs (Harma and Suomalainen 1951) and in rats (LeBlanc and Rosenberg 1957a and LeBlanc 1963a), has been shown to cause an alteration in the number of mast cells in the peripheral and central tissues. These authors did not establish a functional relationship of this change in number of mast cells to their possible function in the animal's response to cold. However, mast cells contain substances with vasodilatory actions that could conceivably be involved in the vascular adjustments made by an animal in its response to cold exposure. Some of these vasoactive compounds include histamine, which is present in the mast cells of all species thus far investigated (Selye, 1965), seratonin, which is present in the mast cells of rats and mice (Bendett _et al._ 1963), and dopamine, which is present in the mast cells of ruminants (Falck et al. 1964).

Histamine levels have been found to increase in rats during cold exposure. Histamine excretion rates tend to parallel changes in mast cell numbers suggesting that mast cell function may be influenced by cold exposure (LeBlanc and Rosenberg 1957a). The effect of histamine on sympathetically constricted vessels has been recently investigated by Steinsland and Hieble (1978) who demonstrated that histamine produces a concentration dependent inhibition of the constrictor response of the isolated ear artery of rabbits to brief intermittent periods of sympathetic stimulation. Carroll and Neering (1976) have also shown that histamine causes vasodilatation in rabbit ear arteries constricted by noradrenaline. Kiernan (1975) found that the dilatation of ear vessels in rats in response to tissue injury can be suppressed by depleting the mast cell granules with the mast cell degranulator, compound 48/80, prior to injury. Rothschild and Oliveria Antonio (1971) found that noradrenaline and adrenaline injected intravenously into rats or cold exposure (10 min at 8C) caused alterations in the morphology of mesenteric mast cells in rats. These authors described these alterations as "reminescent of that induced by the potent synthetic histamine releasing agent, compound 48/80," and interpreted this finding as support for a role of mast cells in modulating the effects of excessive sympathetic pressor activity.

Although in some species there is evidence for an alteration of mast cell number in the skin during cold exposure, the functional significance of this change is not clear. Since histamine is a vasodilatory substance, it is possible that mast cells could be involved in the process of cold induced vasodilatation and, therefore, play a role in preventing tissue damage during cold exposure. The objectives of the following study were to determine the number and distribution of mast cells in skin sample sections of cattle and sheep by inducing monoamine fluorescence by exposure to paraformaldehyde to test the hypothesis that cold environments will induce a change in mast cell numbers in the skin.

MATERIALS AND METHODS

Experiment 1-1: Fluorescent Observation of Mast Cells in the Skin of Cattle

1. Experimental Animals (Trial One)

In experiment 1-1 two trials were conducted to investigate the distribution and number of mast cells in the skin of cattle exposed to a warm (20C) or a cold (-15C) environment. In trial one five mixed breed, beef type, three year old steers (40% Angus, 35 % Charolais, 16 to 19% Galloway, 4 to 5% Hereford and 0.1 to 0.8% Brahman) were used. Three of these animals had been used in an experiment to measure the effects of drinking water temperature on various ruminal parameters during cold exposure. These three animals were stanchioned on rubber mats in a temperature controlled room 3.2 m x 2.9 m, at -15C for 4 wk prior to the collection of skin samples. They were fed at a level of 150% of the NRC (1970) maintenance recommendations. The ration consisted of 50% pelleted sun-cured alfalfa and 50% rolled oats on a dry matter basis. The steers were fed at 0800 hours and 1745 hours daily. Fifteen litres of water were available for 5 min to the animals within 15 min of the morning feeding. All morning rations included Bloat Guard (Salsbury Laboratories Kitchener, Ontario) fed in amounts according to the manufacturers recommendations. Cobalt iodized salt was available to the steers at all times. The remaining two steers were maintained indoors in a 3 m x 3 m pen with sawdust bedding at approximately 20C. They were fed at NRC (1971) maintenance levels with water and cobalt iodized salt blocks available free choice. All animals were housed at the Metabolic Research Unit, Edmonton Research Station, the University of Alberta.

2. Experimental Protocol (Trial One)

At the conclusion of the 4 wk cold exposure two skin samples were taken from each animal, one from the lateral surface of the left upper fore-leg at a point level with the chest and a second from the left mid-side at about the S2 position of Dowling (1955). These samples were taken by means of a coring device described by Newell and Bowland (1971) for obtaining backfat sample cores from pigs. The device is essentially a cork borer constructed of stainless steel with a beveled cutting edge, driven by a 0.65 mm electric drill (Black and Decker Variable Speed B-202-5 Deluxe 1/4 inch Drill, Black and Decker Manufacturing, Brockville, Ontario). It removed a circular skin sample 1 cm in diameter. A stop ring on the coring device was set so that the cutting edge descended until it just penetrated the internal side of the skin as judged by the small portion of loose connective tissue or subcutaneous muscle found on the inner side of the sample, a depth of about 8 mm.

Samples were immediately transferred to disposable tissue containers (28 mm x 5 mm) (Lab-Tek Products Division of Miles Laboratories Inc; Naperville, Illinois) and frozen in isopentane, cooled by liquid nitrogen. The tissue samples were then prepared as follows for examination as described by Cottle and Nash (1974). Tissue samples were freeze dried for 4 d in an Olson freeze drying apparatus at -35C and a pressure of less than 1 x 10^{-3} torr. Dried tissue blocks were exposed to paraformaldehyde for 2 h at 80C followed by embedding in paraffin under vacuum. Sections (10 μ thick) were cut and mounted on slides which had been sparingly coated with Gurr's glycerine albumen and dried. The paraffin was removed by dissolving with xylene and the sections mounted in paraffin oil.

Flourescent histochemical observations were made with a Leitz Ortholux ^{R} microscope with a BG12 filter (4.5 mm thickness) at the U.V. source and a barrier filter of 530 nm. Photographs were taken using Kodak ^{R} Ecta DL film, ASA rating 160, in a Leitz automatic camera. The microscope was adjusted to allow sufficient bright field light to observe other histological structures in addition to the fluorescing mast cells.

Mast cells were counted by observing the resulting 35 mm photographic slide of five independent sections of the 63x magnification on a Singer Caramate projector, model L-880-6 (Singer Education Division, Rochester, N.Y.). A transparent grid, 12 cm x 12 cm, ruled into 2 cm x 2 cm squares was taped on the face of the projector such that one edge of the grid lay along the junction line between the epidermis and the dermis. The fluorescing mast cells which fell beneath the grid were counted. The number of mast cells falling within this area was converted to mast cells/mm² of cross section area by dividing the number of mast cells counted by 0.074 as the area beneath the 12 cm x 12 cm grid represented 0.074 mm² in the tissue. For illustrative purposes plates were made from selected typical 35 mm slides. All plates were produced by Northwest Colour Laboratories, Edmonton, Alberta.

1. Experimental Animals (Trial Two)

In the second trial, skin samples were taken from sixteen 1 year old mixed breed steers purchased from an order buyer, thus, their exact breeding was unknown. They appeared to be of mainly Herefore breeding. Eight of these animals were held outside during the winter. The average temperature over a two week period prior to skin sampling was -5.9C. The remaining eight steers were maintained indoors at approximately 19C. The animals were managed and fed four different feeding levels as described by Gonyou (1977). The feeding levels were 1, 1.3, 1.6 and 2.0 times maintenance. The eight steers inside were housed in the Metabolic Unit, Edmonton Research Station, the University of

Alberta. Each animal had 5.1 m² of floor space. Water was available ad libitum from automatic water bowls. Feeding took place at approximately 1330 hours daily in individual pens (3.6 m²) adjacent to the main pens. All cattle inside and outside, were bedded with wood shavings. Bedding material was provided whenever necessary to insure clean dry bedding areas.

The outdoor animals were initially kept at the Ellerslie Bull Testing Station of the University of Alberta at Edmonton, Alberta, in pens providing 8 m² per steer. A roof covered 65% of each pen and protection from the wind was provided by nearby structures with the exception of winds from the west southwest and the east northeast. Individual feeding stalls were provided. Water was available ad libitum from heated water bowls.

On day 99 (January 20, 1976) of the feeding trial being conducted simultaneously with this work (Gonyou 1977) a fire destroyed the facilities housing the outside group of cattle. The animals were then moved to facilities at the Edmonton Research Station of the Department of Animal Science of the University of Alberta at Edmonton, Alberta. Here, the pens provided 80 m² per animal with a roofed area, wind protection and watering facilities similar to the initial pens. Wood shavings were again used as bedding.

2. Experimental Protocol (Trial Two)

Two skin samples were taken per animal, on February 27, 1976, one from the leading edge of the left ear at a point about 12 cm from the point of attachment of the ear to the head and a second from the middle of the left side about the S2 position of Dowling (1955). The former was taken by means of an ear knotcher of the type commonly used to identify pigs and the latter by means of the coring device described in the first trial.

The biopsied pieces of tissue were prepared for fluorescent microscopy as described for the first trial. Photographs were taken under brightfield illumination using Kodak^{R} Exta DL film, ASA rating 160 in a Leitz automatic camera or in some cases with a Leica microscope camera (Ernst Leitz, Wetzlar Germany). The field photographed was selected such that the epidermis was evident in the field. The microscope was then changed to the fluorescent mode and the same field rephotographed. When the Leica camera was used, brightfield exposures were in the order of 2 to 3 s and fluorescent exposures were in the order of 2.5 min. For illustrative purposes plates were made from selected 35 mm slides. All plates were produced by Northwest Colour Laboratories, Edmonton, Alberta.

Mast cell counts were made by the same procedure as described in trial one except that a 10 x 10 cm grid ruled into 2 cm x 2 cm squares was used on the caramate screen.

This process was repeated on five independent sections from each sampling site. Since the 10 x 10 grid represented 0.0079 mm^2 of the tissue the number of mast cells in the grid area was converted to mast cells/mm^2 of tissue by dividing the number of mast cells counted by 0.0079. The number of mast cells/mm^2 of tissue cross section was analysed by analysis of variance (Snedecor and Cochran 1967).

Experiment 1-2: Fluorescent Observation of the Mast Cells in the Skin of Sheep

In order to compare the number of mast cells in the skin of sheep with the skin of cattle, samples were obtained from two sheep.

1. Experimental Animals

Two Suffolk x Southdown crossbred wethers, numbers 590 and 595, weighing 41.7 kg and 39.1 kg, respectively were used. Both animals were housed in metabolic cages, 52 cm by 154 cm, with solid fiberglass sides, an expanded metal floor and dexion frame (Redi Rack Industries, Hamilton, Ontario), 77 cm above the floor. The animals were unable to turn around, however, they were free to stand or lie and move about two steps backward or forward. Animal 590 and 595 were housed at 0C and 20C, respectively, for 2 wk in a temperature controlled room at the Metabolic Research Unit, Edmonton Research Station, the University of Alberta.

Both wethers were fed a maintenance ration including salt and minerals but no salt blocks or mineral blocks were available to the animals. Feed and water were available to the sheep between 1400 and 1600 hours daily. The sheep were maintained under constant light.

2. Experimental Protocol

On the day of the experiment the sheep were removed from their metabolic cages and killed by injection of a saturated solution of magnesium sulphate into the jugular vein. Skin samples (about 5 mm x 5 mm) were immediately excised from the left ear and the left mid-side over the ninth to tenth rib. These samples were prepared for fluorescent observation as described in experiment 1-1.

The mast cells were counted on individual sections from the tissue samples as described in the second trial of experiment 1-1.

RESULTS AND DISCUSSION

Mast cells appeared in definite distribution patterns throughout the skin sections of sheep and cattle. They could be observed throughout the dermis although they appeared to be mostly located in the papillary layer (i.e. from the epidermal dermal junction to just below the hair follicles). The number of mast cells progressively declined towards the deeper (reticular) layers of the dermis. They appeared as brightly fluorescing yellow points due to their dopamine content. No mast cells were found in the epidermis of any of the skin sections examined (see plate 1).

Plate 1 shows a typical cross section (217x) of skin taken from the upper leg of a cold exposed (-15C) steer in experiment 1-1. The dermal epidermal junction is evident toward the upper left corner of the photograph. The stratum corneum can be seen on the outer edge of the epidermis slightly pulled away from the rest of the section. Four hair follicles cut in longitudinal or oblique sections are evident. Mast cells appear to be present in groups (A) or in linear arrangements (B) in this plate. Mast cells were frequently observed adjacent to the structures of the skin such as the sebaceous glands, hair follicles, and sweat glands.

In plate 2 which is also a cross section of the

Plate 1: Fluorescence photomicrograph of a skin section (217x) from the upper forelimb of a cold exposed (-15C) steer. (Trial one). The epidermis is at the upper left.

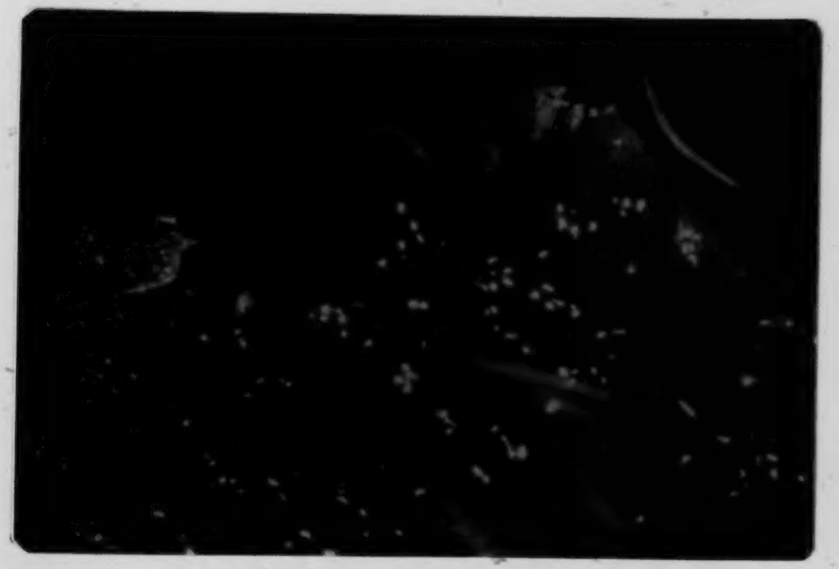
(A) Mast cells arranged in group

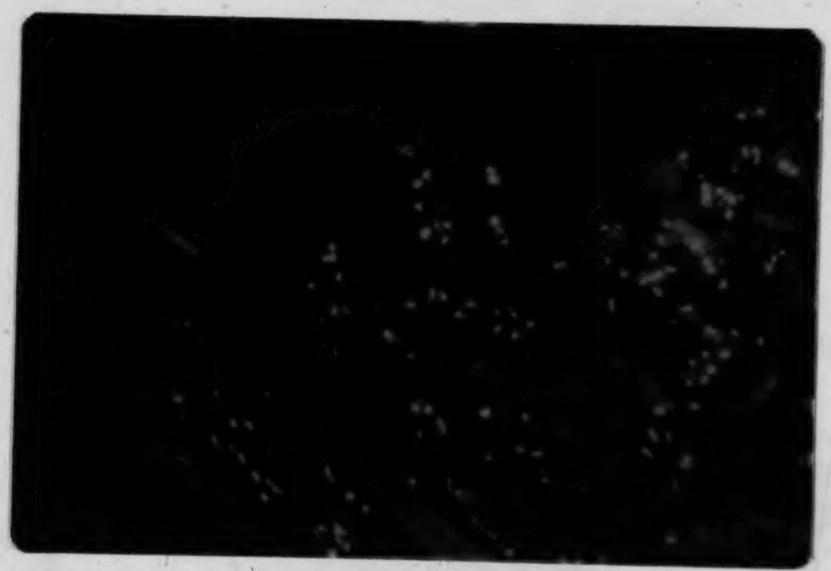
(B) Linear arrangement of mast cells

(C) Mast cells adjacent to a blood vessel

Plate 2: Fluorescence photomicrograph of a skin section (217x) from the upper forelimb of a cold exposed (-15C) steer. (Trial one).

(A) Mast cells surrounding a tubular structure cut in longtitudinal section (probably an arteriole or artio-venous anastomosis).





extremity skin of a cold exposed (-15C) steer the definite patterns of the mast cells is evident. Of particular interest in this section is the area at (A) in which the mast cells appear to surround a hollow, curved tubular structure cut in longitudinal section. This is probably a cutaneous blood vessel (most likely an artericle cr arterio venous anastomosis, judging from its diameter of approximately 20 µm).

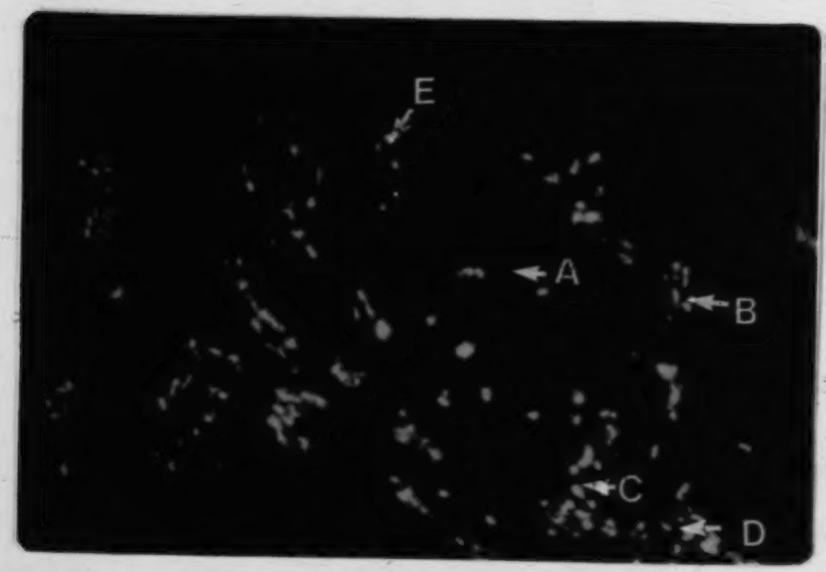
Plate 3 is a cross section through the leg skin (217x) of a warm exposed animal with less bright field illumination than plates 1 and 2. In this plate there are not only mast cells, but also fine lines of fluorescence (A) which are probably adrenergic nerve fibers. Again the mast cells are not spaced at randcm throughout the section but can be seen in aggregations (B), circular patterns (C), and linear patterns (D). Of particular interest are the three cells seen at E in plate 3 which appear to have fluorescing processes extending from them. A higher magnification (546x) of this area is seen at (A) in Plate 4. These cells can be seen to surround a small circular object, probably an arteriole or venule cut in cross section with a slightly fluorescing luminal surface. The processes extending from the mast cells can be seen to almost surround the vessel. Again, in this photograph the fine fluorescing lines of adrenergic nerve fibers can be seen (B). Several fibers

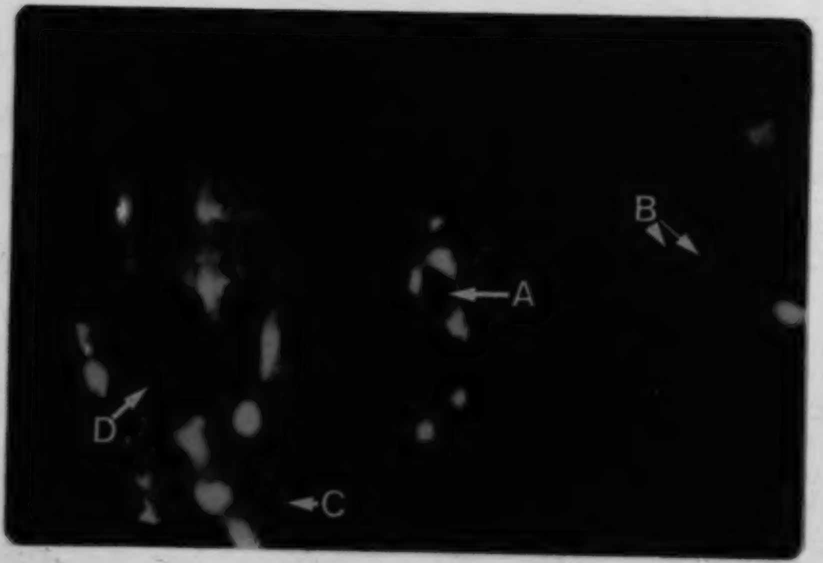
Plate 3: Fluorescence photomicrograph of a skin section (217x) from the upper forelimb of a warm exposed (20C) steer. (Trial one).

(A) Adrenergic nerve endings
(B) Mast cells arranged in a group
(C) Circular arrangement of mast cells
(D) Linear arrangement of mast cells
(E) Mast cells surrounding a circular structure cut in cross section (probably an arteriole or venule).

Plate 4: Fluorescence photomicrograph of a skin section (549x) from the upper forelimb of a cold exposed (-15C) steer. (Trial one).

(A) Mast cells surrounding a circular structure (probably an arteriole or venule) cut in cross section
(B) Adrenergic nerve fibers
(C) Varicosities of the adrenergic nerve fibers


E
A
B
C
D


B
A
D
C

show prominant vericosites (C). The area in the lower right hand corner of the figure may be part of a hair follicle sheath but it is difficult to identify with certainty the structures receiving adrenergic inervation. The structure at the lower left hand corner of fig. 4(D) may be a muscle bundle or errector pili muscle fibers with adrenergic innervation (C).

Plate 5, which is a bright field photomicrograph (549x) of a section from an ear of a steer kept indoors throughout trial two, is taken relatively deep within the ear as the central supporting cartilage of the ear appears on the right hand side of the plate. Of the four blood vessels to the left of the centre, one has a smaller lumen and a thicker wall than the other three, suggesting one artery and three veins. This would perhaps suggest evidence for a counter current heat exchange mechanism in the deep blood vessels of the ears of cattle. This observation is in agreement with Goodall and Yang (1954) whose diagrams depict close apposition of arteries and veins and who report widely distributed venae comites throughout the skin of Ayrshire calves and embryos.

Plate 6 shows the same area as plate 5 under fluorescence microscopy. The fluorescence due to sympathetic innervation can be seen surrounding three of the blood vessels and traces of the innervation surrounding the

Plate 5: Brightfield photomicrograph of a skin section (549x) from the ear of a warm exposed steer showing an artery and three veins (Trial two).

Plate 6: Fluorescence photomicrograph of the same skin section (549x) shown in plate 5 from the ear of a warm exposed steer showing the adrenergic nerve endings surrounding the vessels. (Trial two).

(A) Varicosities on the adrenergic nerves

A

fourth can also be distinguished. The vericosities on the nerve can be seen in the brightest fluorescing area of the lower right blood vessel (A).

Plate 7 is a photomicrograph (549x) of a mid-side skin section of an animal which had been kept outdoors during trial two. The epidermis appears at the upper right hand margin of the picture. Of particular interest is the inverted U shaped figure (A) which has been outlined with dotted lines. This appears to be a longitudinal section through a tubular shaped structure. No hair follicles are seen in the section, thus it is unlikely that the tubular structure is a sweat gland or sebaceous gland duct as Benjamin (1971) found these invariably associated with hair follicles in the skin of cattle. It probably respresents a portion of one of the tortuous blood vessels found within the skin as described by Montagna and Parakkal (1974). The majority of the mast cells in plate 8, a fluorescence photomicrograph of the same section shown in plate 7, can be seen to be located around the blood vessel (A). This agrees with the observations of LeBlanc and Rosenberg (1957a) and Selye (1965) of other species in which skin mast cells were observed to be frequently located close to blood vessels. In the wall of the vessel, there appear to be adrenergic nerve fibers with varicosities. However, these appear very faintly. The diameter of the vessel is approximately 20 µm

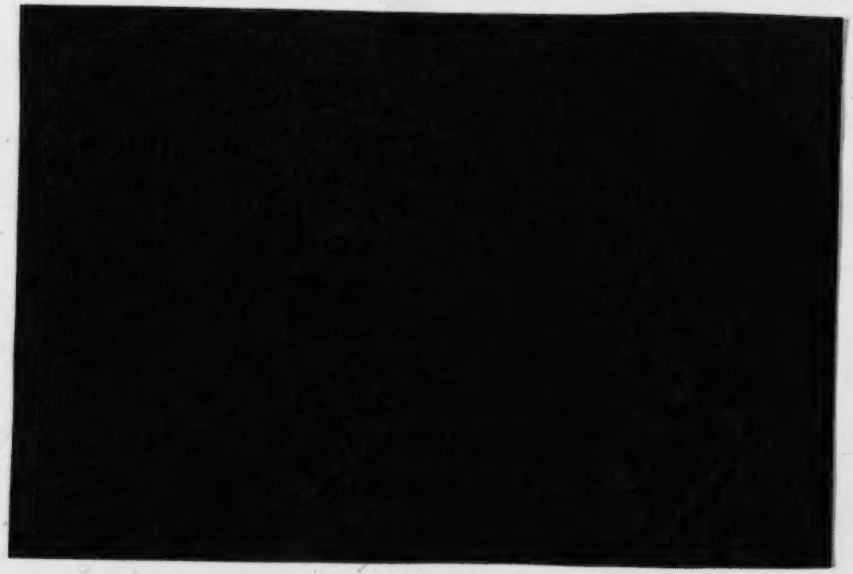
Plate 7: Brightfield photomicrograph (549x) of a mid side skin section of a steer exposed to a cold environment (Trial two). Epidermis at the upper right.

(A) Longitudinal section through a "horseshoe" shaped tubular structure (probably a blood vessel) outlined with a dotted line.

Plate 8: Fluorescence photomicrograph (549x) of a mid side skin section of a steer exposed to a cold environment (Trial two).

(A) Longitudinal section through a "horseshoe shaped" tubular structure (probably a blood vessel) surrounded by mast cells.

7



A

and therefore probably represents an arteriole, venule or arterio-venous anastomosis.

The number of mast cells in skin from the mid-side and the extremity from the five cattle utilized in trial 1 are given in Table 1. No statistically significant differences ($P<0.05$) existed in the number of mast cells between the extremities and the mid-side or between the cold and warm exposed animals. The lack of statistical significance in this observation may have been due to the small number of samples available for observation.

The number of mast cells/mm^2 cross section area of skin for trial two are given in Table 2. Again no significant differences were found, due to indoor or outdoor housing of the animals or to their plane of nutrition. Mast cell numbers were higher in trial two than in trial one. This difference is likely due to the fact that in trial two the area counted did rot extend as deeply into the dermis where the number of mast cells is less as shown in Plate 9.

The results of these experiments suggest that cold exposure in the breed of cattle examined does not alter the mast cell numbers in the skin of the trunk or extremities. This is contrary to the findings in man by LeBlanc _et al._ (1960) and in rats by LeBlanc and Rosenberg (1957a). LeBlanc (1963a) reported an increase in abdominal skin mast

Table 1. The mean number of mast cells per mm^2 cross-section of skin mid side of warm and cold exposed steers. Experiment 1-1, trial 1.

	Cold (-15C)	Warm
No. of animals	3	2
No. of mast cells/mm^2		
Mid side	926	729
Leg	1354	1024
SE[1]	93.2	

[1] SE-Standard error of the means.

Table 2. The mean number of mast cells per mm^2 cross-section of skin from the ear and the mid-side of indoor and outdoor groups of steers. Experiment 1-1, trial 2.

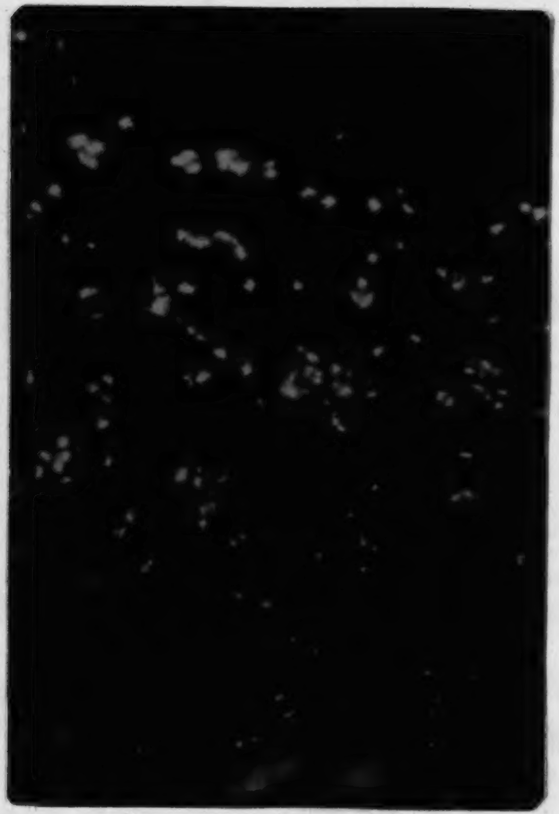
		Ration[1]				
		1x Maintenance	2x Maintenance	3x Maintenance	4x Maintenance	SE[2]
Indoor	Ear	1576[3]	1528	1577	2109	
	Mid side	2123	1405	1690	1898	
Outdoor	Ear	1973	2672	1572	1403	
	Mid side	1506	2317	1690	1309	47.0

[1] Maintenance rations determined according to NRC recommendations, 1971.

[2] SE-Standard error of the means.

[3] Two animals per observation.

Plate 9: Fluorescence photomicrograph of a skin section (217x) from the upper forelimb of a cold exposed (-15C) steer. (Trial one). Epidermis at the top of the photograph showing the decline in mast cell numbers from the more superficial to the deeper layers of the dermis.



cell concentrations from 84 ± 20 cells/mm² to 134 ± 12 cells/mm² in rats exposed to 23C and 6C for 2 wk, respectively. Abdominal skin from rats exposed to 2C for 4 wk were found to have a mast cell concentration of 124 ± 6 cells/mm². In the ear skin of these rats, the number of mast cells declined slightly from 31 ± 5 cells/mm² to 23 ± 6 cells/mm² after 2 wk at 6C and to 13 ± 3 cells/mm² after 2 wk at 2C.

In experiment 1-2, four independent sections were counted from each of the tissue samples taken from both sheep. The number of mast cells/mm² per cross sectional area were 634 ± 535 for the ear and 684 ± 376 for the mid-side of sheep number 590 housed at 0C and 654 ± 233 for the ear and 337 ± 73 for the mid-side of sheep number 595, housed at 20C. The data indicate that the cold environment may have increased the number of mast cells in the mid-side of the cold exposed sheep, however, a greater number of sheep would have to be studied to arrive at definite conclusions. No apparent difference existed in the mast cell numbers in the extremities. The number of mast cells found by the fluorescent technique was smaller than that found in experiment 1-1 for cattle but still larger than those reported by LeBlanc and Rosenberg (1957a) in rats. The number of mast cells in this study is not consistent with the report of Vegad (1970) who observed by means of

Liechman's staining technique (Culling 1963) that the number of mast cells was less in sheep than that seen by him in rats and mice. However he did not state a specific number for mast cells in sheep, rats or mice. He also stated that mast cells in sheep were difficult to stain. Perhaps he was able to only stain a certain percentage of the population thereby under estimating the total population.

The large number of mast cells in ruminant tissue (>330/m² skin) observed in the present study confirms the studies of Coupland and Heath (1961) who reported 534 ± 54 cells/mm² in the capsula hepatis of cattle compared to 55.8 ± 7.6 cells/mm² for that of the horse. Riley and West (1952) provide the only other counts of mast cells in the ear skin of cattle. Unfortunately the mast cell counts are given per "microscope field" which was undefined, making it impossible to compare the number given with the number found in this work.

Since the number of mast cells in the skin of cattle and sheep is large to begin with, the lack of an increase in the number in the outdoor group of cattle and in the cold exposed cattle and sheep does not necessarily mean that mast cells have no function in the response of cattle and sheep to cold. Mast cells were observed in close proximity to the blood vessels of the skin and in greater numbers towards the more superficial layers of the skin. These facts suggest a relationship might exist between mast cell function, the environment of the animal and the peripheral blood flow. There could well be changes in release of mast cell constituents without a change in mast cell numbers. Since the mast cell numbers in sheep were almost as large as those seen in cattle and because of the considerably greater ease with which sheep can be utilized for infusion experiments, sheep were utilized in the experiments reported in Chapter two and Appendix 1 to determine a physiological role of histamine in control of blood flow with particular reference to cold.

Chapter 2 - Effects of Histamine, Histamine Releasers and Histamine Blocking Agents on Peripheral Blood Flow and Skin Temperature.

LITERATURE REVIEW

Physiologists have been interested in histamine's potent vascular effects and hence its possible participation in the control of the cardiovascular system almost since the first laboratory synthesis of histamine by the decarboxylation of histidine by Windaus and Voght in 1907 (Dale and Laidlaw 1910). In the first description of the pharmacological actions of histamine Dale and Laidlaw (1910) reported that it was a vascular dilator in the limbs of dogs and cats. It has not been conclusively determined whether endogenous histamine participates in the control of the peripheral circulation in conditions where tissue trauma does not exist. Evidence exists that histamine, possibly from mast cell origin, may play a role in the control of peripheral circulation. Selye (1965) notes "the well known tendency of MC "(mast cells)" to arrange themselves in close contact with the walls of small blood vessels". In sheep (Vegad, 1970) and in cattle (Chapter one of this thesis) mast cells have been reported in close proximity to blood vessels. Riley and West (1952) identified mast cells as a major endogenous source of histamine in cattle, pigs, and rats, by correlating the number of mast cell with the histamine content of the tissues. Anderson and Uvnas (1975) used autoradiography of rat peritoneal mast cells labelled by incubation with ^{3}H-histidine to locate histamine within the cytoplasmic electron dense granules characteristic of mast cells.

Compound 48/80, a condensation product of p methoxyphenethyl-methylamine and formalin, which is effective in producing mast cell degranulation in various species including sheep (Vegad 1971a and b), has been widely used to investigate the effect of mast cell histamine on blood vessels. Kiernan (1972) demonstrated that vasodilatation occurred concurrently with degranulation of mast cells at the site of an injury. He found this vasodilatation in the ears of rats could be suppressed (Kiernan 1975) if the mast cells had previously been depleted of granules by compound 48/80. Howland and Spector (1972) found that the specific mast cell degranulation compound 48/80 markedly reduced the histamine content of rat vascular tissue and concluded that such histamine was stored in the mast cell. On the other hand Adams and Hudgins (1976) found that compound 48/80 did not enhance the loss of radioactivity in a rabbit aorta previously labelled with ^{14}C-histamine. This finding may be questioned as they removed the connective tissue surrounding the aorta which may have contained the majority of the mast cells. Thus the source of the histamine that may be involved in vascular reactions is still a matter of contention.

Urinary and tissue histamine levels have been shown to respond to environmental temperature. An increase in mast cell numbers in hibernating hedghogs was reported by Harma and Soumalainen (1951); an increase in some tissues and a decrease in others was confirmed in man by LaBlanc et al. (1960) and in rats by LeBlanc and Rosenberg (1957a and b). These changes in mast cell numbers have been shown by LeBlanc (1963b) to be concurrent with an increased urinary histamine excretion. Fujieda (1975) has found that acute cold exposure (-10C for 60 min) causes an increase in histamine content of the rat liver, where as no change was found in the pinna of the ear or in interscapular brown adipose tissue. Chronic cold (5C for two weeks) however, caused a marked increase in the histamine content of the brown-adipose tissue, a moderate increase in the liver, and a decrease in the pinna of the rat. The possible relationship of these changes in histamine content to the increased blood flow to these organs in a cold environment as shown by Evonuk and Hannon (1963) in the rat liver and by Alexander et al. (1973) in lamb brown adipose tissue was not discussed by Fujieda (1975).

Cold exposure also alters an animals response to exogenous histamine. Histamine (32.4 mg/rat) injected into

150 g rats exposed to 21C, but previously housed for 28 d at either 6C or 28C, resulted in a decline in rectal temperature (LeBlanc and Rosenberg 1957b). The decline in the group housed at 6C for 28 d was less than that in the group housed at 28C suggesting to LeBlanc and Rosenberg (1957b) that cold exposure diminished the response of the animals to histamine. LeBlanc (1963c) also found that the blood pressure drop in response to injected histamine (0.1 µg to 5.0 µg) declined over the first three months of cold exposure (6C) and then returned to normal by the end of one year. These results indicated to him that there may have been a decline in the vascular sensitity to histamine during the first three months of cold exposure when urinary histamine was at its peak.

Catecholamines released from the sympathetic neurons and the adrenal medulla may influence the release of histamine from tissues. Noradrenaline and adrenaline are both known to be excreted in the urine of rats in larger amounts in the cold (Cottle 1960, Leduc 1961a and LeBlanc and Nadeau 1961). This increase is also seen in sheep as reported by Webster _et al._ (1969). The plasma concentration of catecholamines has also been shown to be increased several fold in sheep during acute cold exposure (Thompson _et al._ 1978). Szilagyi _et al._ (1960) found that adrenaline injected into the saphenous vein in normothermic dogs elevated plasma histamine concentration provided rectal temperature did not drop below 21.5C. Coppola and Di Palma (1962) found that noradrenaline injected intravenously also increased the blood level of histamine in dogs. Tuttle and McCleary (1970) reported that stimulation of the sympathetic nerve ~~to the~~ isolated perfused gracilis muscle of the cat increased the outflow of radioactively labelled histamine from the muscle. Thus the increased sympathetic activity which occurs during cold exposure may serve to increase the amount of histamine released from tissues of a cold exposed animal.

It is not known whether the source of the histamine released in response to catecholamines is the mast cell or some other cell type. Whelan (1956) could not detect an increased outflow of histamine during the vasodilatation of a cold exposed hand. He felt that this may have been due to the insensitivity of the bioassay available at the time. However he did conclude that if histamine did participate in vasodilator actions it must be formed within the cells on which it acts (the muscle cells of the blood vessels) and that these cells must be impermeable to antihistamine substances as the antihistamines known at that time could not completely abolish blood flow responses to exogenous histamine.

On the other hand, evidence exists for a source of histamine outside of the smooth muscle cells of the vascular system. Rothschild and Oliveira Antonio (1971) reported that sympathetic nerve stimulation, adrenaline, and noradrenaline injections and exposure of rats to cold (8±0.5C for 10 min) all produced an increase in the proportion of "altered" mast cells. This alteration of the mast cells was not identical to the degranulation they observed in response to compound 48/80, however, they felt it was a less intense manifestation of the same mechanism. They also found that L-adrenaline was capable of releasing histamine from isolated mast cells. The ability of noradrenaline to release histamine was not investigated. They also found that adrenaline could produce the same type of altered mast cell, a response that could not be produced by isoproternol, an amine acting solely on β receptors and which could be blocked by α sympatholytic agents. This suggested to them that an α type receptor existed on mast cells. They suggested a role for mast cells in counteracting the effects of "excessive sympathetic pressor activity". Heine and Forster (1974) state on the basis of observing "the termination of the most delicate endings of postganglionic neurons in the cytoplasm of mast cells" that mast cells are connected to the autonomic nerve endings. Thus mast cells may also be a source of the dilator histamine released by catecholamines.

A portion of the early difficulty in investigating the physiological role of histamine stemmed from the inability of the classical antihistamine drugs such as mepyramine and benadryl to block all of the responses to histamine. Folkow et al. (1948) noted that if small doses of histamine (0.5 µg) were given to cats and dogs, neoantirgan (1 mg/kg) completely blocked the vasodilatory response. If however 5 µg of histamine was given, 25 mg/kg of the antagonist did not reduce the vasodilatory response. They proposed on the basis of these observations two different receptor sites for histamine. One receptor site at which the classic antihistimines competed with histamine and one site at which they did not. Ash and Schild (1966) designated the responses to histamine which could be blocked by the classic antihistamines H_1 receptors. Black et al. (1972) found that responses to histamine in the rat such as increased acid secretion by the stomach increased heart rate and inhibition of uterine contraction which could not be antagonized by mepyramine and other H_1 receptor blockers could be blocked by burimamide ^{R} and metiamide (Black et al. 1972). These responses they suggested were due to a second separate histamine receptor designated the H_2 receptor. These two forms of histamine receptor have been extensively studied in many tissues of the body and in many species and the results reviewed by Chand and Eyre (1975).

The availability of the H_2 blockers burimamide and metiamide has lead to the identification of both of H_1 and H_2 types of receptors in the vascular system. Powell and Brody (1973, 1976a) found that the fall in blood pressure in anesthetized dogs and cats was only partially attenuated by mepyramine. Further treatment with burimamide ^{R} caused nearly complete attenuation of the response to histamine. In the gracilis muscle of the dog burimamide ^{R} alone had no effect on histamine induced vasodilatation but further attenuated the reduced vasodilatation which occurred after mepyramine. In the intact dog intravenous mepyramine prevented the increased heart rate following histamine but not the inotropic effects. Further administration of burimamide prevented the negative inotropic effect of histamine. From these results they concluded that both peripheral vasculature and cardiac histamine responses were mediated through H_1 and H_2 receptors. They also reported (Powell and Brody 1976b) that neither H_1 blocking, H_2 blocking or a combination of both modified post occlusion vasodilatation or post exercise dilatation. They found however that both blockers given in sequence reduced reflex vasodilation in the perfused hind limb in response to increased arterial pressure suggesting both receptors may be involved in this response. The post stimulation (of the sectioned lumbar sympathetic chain) dilatation of the perfused gracilis muscle could be totally reduced by mepyramine suggesting that only H_1 receptors participated in this response. In humans, Duff and Whelan (1954) reported that mepyramine antagonized the dilatation caused by intra-arterial histamine in the forearm. Recently, however, Chipman and Glover (1976) ammended the dilatory action of histamine on forearm blood flow to include H_2 receptors which appeared to account for the delayed return of vascular tone to preinfusion levels. The constriction of the rabbit ear artery in response to histamine has been considered an H_1 receptor response (de La Lande and Rand 1965) which is in agreement with Powell and Brody (1976b) who found that the constriction of the saphenous vein in dogs in response to histamine could be completely attenuated by H_1 blockade. The dilatory response which occurs in rabbit ear arteries after the H_1 constrictor response to histamine has been blocked was found by Glover et al. (1973) to be greatly reduced by burimamide and was considered to be an H_2 response. Angus et al. (1977) have identified both H_1 and H_2 receptors in the hind limb vessels of the rabbit.

Both H_1 and H_2 receptors have been identified in the tissues of sheep. Eyre (1973) found that the histamine induced relaxation of the bronchi which had previously been reported to be resistant to inhibition by mepyramine (Eyre 1969) could be blocked by burimamide and hence was attributed to H_2 receptors. On the other hand, Brigham et al. (1976) found that the histamine induced pulmonary edema in sheep could be entirely blocked by diphenhydramine, an H_1 blocker, while metiamide did not affect the response. Thus, within the same organ of an animal different tissues appear to have different populations of histamine receptor types. Thus differing smooth muscle groups respond differently to similar doses of histamine.

There appears to be a relationship between the ability of histamine to dilate a vessel following H_1 blockade and the mechanism cf constriction of the vessel. Carroll and Neering (1976) found that the H_2 receptor dilaticn response to histamine was greater in the rabbit ear artery if the artery had been previously constricted with noradrenaline than if it had been constricted by caffeine or barium. This may suggest a relationship between the sympathetic constriction of blood vessels and the reaction of histamine with blood vessels.

Heitz and Brody (1975) found that they could block the dilatation of the vascular system of the gracilis muscle that follows electrical stimulation with the antihistamine tripelenamine. They found that prior constriction was not necessary to elicit this response as the dilatation could still be demonstrated after stimulation in the presence of xylocholine. This dilatation however could be blocked by phentolamine, an α receptor blocking agent, even though the adrenergic vasoconstrictor tone was absent. They suggested from these results that (1) the release of histamine from its storage site is mediated by an α receptor mechanism in agreement with Rothschild and Oliveira Antonio (1971) and; (2) that the histamine release may be under the control of adrenergic fibers which do not have a vasoconstrictor function.

Beck (1965) found that triplenamine, an H_1 receptor antagonist, blocked the active component of reflex vasodilation in the perfused hind limb of the dog which had been constricted by adrenaline or noradrenaline. He postulated a histaminergic vasodilator system which produced an active dilatation of vessels constricted by the sympathetic nervous system. Brody (1966) was unable to demonstrate the release of histamine by the fluorescent method of Shore *et al.* (1959) during reflex vasodilatation which he attributed to the rapid and efficient uptake and breakdown of histamine by the tissues. He was, however, able to demonstrate increased plasma ^{14}C-histamine and ^{14}C-methylhistamine associated with the reflex vasodilatation caused by intravenous noradrenaline and by stimulation of the carotid nerve. These experiments demonstrated that isotopically labelled histamine could be taken up into anatomical sites, which he was unable to define, and liberated by neurogenic stimuli during the active phase of reflex vasodilatation. He suggested that these storage sites were either the sympathetic nerve terminals or sites near the nerve terminals. Thus, histamine, regardless of its source, appears capable of causing an active dilatation of vasculature beds constricted by the sympathetic nervous activity.

The receptor sites that have been identified in the cardiovascular system undoubtedly play a role in mediating vascular responses to histamine released by tissue trauma or injury as described by Kiernan (1972, 1975) in the ears of rats. But, as seen above, they may also play a role in non-pathological situations as regulators of the cardiovascular system during maintenance of homeostasis. Schayer (1965, 1968) has suggested that histamine is the "intrinsic dilator" in what he describes as "autonomous microcirculatory phenomena". This theory proposes that the histamine produced within the smooth muscle cells by histidine decarboxylase dilates the precapillary sphincter. Histidine decarboxylase is an inducible enzyme as Schayer (1962) found its activity to be greater in the lungs of rats and mice housed in a cooler environment (23-24C) compared to a warmer (36-37C) environment. This suggests a participation of histamine in the animals response to its external environmental temperature.

In spite of the mounting evidence that histamine can cause active dilation of the vasculature constricted by sympathetic nervous activity, few experiments have been conducted on the relationship of histamine to the dilatation of the peripheral vasculature in the cold. Duff _et al._ (1953) induced dilatation of warm (29C) fingers but not in cold fingers (0-2C) in response to arterially infused histamine. They, however, questioned the amount of histamine being delivered to the cold constricted periphery. Oral doses of benadryl did not affect the response to immersing the index finger in cold water. Whittow (1955), however, found promethazine hydrochloride, another oral antihistamine, significantly reduced the cold induced vasodilation and delayed the onset of the response. When promethazine hydrochloride was introduced by iontophoresis, an increase in the magnitude of cold induced vasodilation was seen which was attributed to the local anesthetic effect of the drug seen by other investigators (Halpern _et al._ 1947 and Greenfield _et al._ (1952). The decrease in cold induced vasodilatation caused by the oral dose of promethazine hydrochloride was attributed to the central depression effect of this drug as seen by Winter (1948) and Glaser (1953). However, in these experiments only one dose level for each of the drugs studied was reported. No evidence for either a local anesthetic or for a central depression caused by these levels of the drugs was presented. Schwinghamer and Adams (1969), found no reduction in the cold induced vasodilatory response when diphenhydramine hydrochloride (10 mg/kg) was given to anesthetized cats 30 min prior to cooling the hind paw. Woods *et al.* (1976), however, reported that diphenhydramine hydrochloride prevented the increase in blood flow in the uterus of sheep for only 10 min after its injection. Thus the effect of the diphenhydramine hydrochloride in the experiments of Schwinghamer and Adams (1969) may have been declining by the time cold exposure occurred.

Thus, inspite of the negative results reported by the above authors a number of points may be summarized which suggest a possible role for histamine in the control of peripheral circulation in a cold environment. These points are as follows: (1) histamine excretion and tissue mast cell numbers in rats increase dramatically in some tissues in the cold; (2) the vascular system has been shown to have specific receptor sites (designated as H_1 and H_2 receptors) for histamine; (3) the peripheral vasculature in several species is capable of dilating in response to histamine; (4) catecholamines, which are released during cold exposure, appear to influence the release of histamine, (5) histamine has been implicated in the reactive hyperemia which follows catecholamine induced vasoconstriction. Therefore, the following series of experiments was designed to further investigate whether histamine could induce dilatation of the peripheral vasculature in cold exposed sheep and whether histamine blocking agents could prevent the "hunting response" in these animals.

MATERIALS AND METHODS

Experiment 2-1: The effect of histamine blocking agents on the ear surface temperature of cold exposed sheep following injection of histamine-HCl

1. Experimental Animals

Experiment 2-1 was conducted using one Suffolk and two Cheviot adult wether sheep weighing 87.3±8 kg. This experiment consisted of a total of eight trials, three conducted with each of the Cheviots and two conducted with the Suffolk in which the response of ear surface temperature to subcutaneous histamine-HCl was measured. The ability of the histamine blocking agents benadryl and metiamide to modify any temperature response to histamine-HCl was also studied.

The sheep were maintained in an indoor pen at a room temperature of approximately 20C. The sheep each consumed 1000 g of dehydrated alfalfa pellets per day, fed in two equal portions at approximately 0800 and 1600 hours. The second portion of the feed was fed at the end of the trial if the trial extended later than 1600 hours. On the day of a trial the experimental animal was not fed the 0800 hour portion of feed and preparation of the animal for the experiment began at this time. All animals were housed at the Metabolic Research Unit, Edmonton Research Station, The University of Alberta.

2. Catheterization

An indwelling Tygon^{R} catheter (Tygon^{R} microbore tubing Norton Plastics and Synthetics Division, Akron, Ohio) 1.0 mm internal and 1.8 mm external diameter, was inserted through a 12 gauge hypodermic needle into the jugular vein 15 cm in the direction of the heart. The catheter was filled with physiological saline containing 500 I.U./ml of heparin (Hepalean, Heparin disodium, 10,000 Heparin units/ml, Harris Laboratories, Brantford, Ontario).

Two additional small Tygon^{R} catheters, 0.25 mm inner and 0.76 mm external diameter 3.7m long were prepared. One end of each catheter was fitted over a hubless hypodermic needle (26 gauge) which could be readily inserted through the skin on the dorsal surface of the ear. One of these catheters was filled with freshly prepared physiological saline containing 0.2 mg/ml Evans Blue dye (C.I. No. 23860, Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawns, New Jersey). The other catheter was filled with a freshly prepared solution of physiological saline containing 20 mg/ml histamine-HCl (Lot 85C 030 Sigma Chemical Company St. Louis, Missouri) and 0.2 mg/ml Evans Blue. One μl of undyed physiological saline was drawn into the end of the catheter to prevent premature contact between the histamine-HCl solution and the animal tissue after insertion of the catheter. Each of the two catheters was clamped with a small hemostat near the junction with a 50 µl syringe to prevent movement of the catheter contents during the installation of the catheters. The ends of the catheters containing the 26 gauge hypodermic needles were passed via a port hole into the temperature controlled room. A needle driver was used to insert a catheter under the skin of the dorsal surface of each ear about 5 cm from the tip of the ear.

3. Temperature Recording

A 36 gauge thermocouple (Thermoelectric Canada Ltd., Brampton, Ontario) was attached to the mid dorsal surface of each ear about 5 cm from the tip of the ear. These were positioned so that the junctions lay on the ear surface above the tip of the hypodermic needle. The thermocouples were held in place by contact cement (Wendar Adhesives, a division of Le Pages Ltd., Edmonton, Alberta) and covered with a single layer of adhesive tape (50 mm x 25 mm). The thermocouples were passed from the sheep via a port hole to a sampling and recording station located outside the temperature controlled room. Both thermocouples were attached either to a Honeywell Model 19 mV recorder (Honeywell Canada Ltd, Toronto, Ontario) set at 2 mV full scale which used an ice bath as a zero reference for calibration or a Honeywell model 15 multipoint temperature recorder (Honeywell Canada Ltd., Toronto, Canada).

Rectal temperature was monitored using a Telethermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio). The thermistor probe was inserted 120 mm into the animals rectum and was held in place by two elastic straps connected to a web strap around the animals thorax.

Room temperature was monitored by a mercury in glass thermometer. This thermometer was suspended in the temperature controlled room adjacent to the observation window.

4. Heart Rate Recording

In order to install electrocardiogram electrodes three 70 mm x 50 mm areas of the animals thorax were closely clipped and the central 30 mm x 30 mm portion of each area was clean shaven. One of these areas was on the dorsal surface of the animal between 60 mm and 120 mm to the right or left of the spinal column, adjacent to the fifth or sixth thoracic vertebrae. The remaining two areas were on the chest immediately behind the left and right fore-limbs. The concave surface of the electrodes in contact with the animal was liberally filled with a conduction enhancing gel (Redox Paste, Hewlett-Packard Medical Electronics, Waltham, Massachusetts). The electrodes were held in place by contact cement and strips of adhesive tape 50 mm x 100 mm) and secured in place by wrapping the entire girth of the animal two or three times with strips of adhesive tape 50 mm wide. This insured good contact with the animal and prevented movement of the electrodes. The cable joining the three electrodes was passed out of the temperature controlled room through a port-hole to a sampling and recording station and attached to a Sanborn Model 7714A Physiological Recording System (Hewlett-Packard Ltd., Toronto, Ontario) for intermittent recording of the heart rate.

5. Restraint of Sheep During Trials

In order to facilitate the installation of the catheters under the ear skin the experimental animal stood in a portable restraining cage with a head stall which limited the lateral movement of the head to an angle of 140 degrees. The animal remained in this restraining cage throughout the trial.

6. Experimental Protocol

In order to allow the animal to recover from any stress caused by the installation of the apparatus the doors to the temperature controlled room were closed and the sheep left undisturbed for a minimum of 30 min at 22 to 23C prior to the start of experimental measurements. The temperature controlled room was then cooled to 1.5 ±2C. Once the temperature had stabilized in the cool environment, 26 μl of physiological saline containing the histamine-HCl was injected via one subcutaneous catheter. Preliminary experiments showed that ear skin temperature markedly increased after injection of 500 µg of histamine-HCl, but dose rates ranging from 10 µg to 200 µg had no effect on ear skin temperature. Thus the 500 µg dose rate was used in all trials reported herein. Control injections of 26 µl of physiological saline were similarly administered into the opposite ear during five of the trials.

When the skin surface temperature had returned to the pre-injection level, following histamine-HCl, an H_1 receptor blocking agent, diphenhydramine hydrochloride (benadryl, Park Davis and Company Ltd., Brockville, Ontario), was injected at a dose of 0.2, 0.375, 0.5 or 0.75 mg/kg via the jugular catheter, over a period of 14 ±1.7 min. A second 500 µg (25 µl) injection of histamine-HCl was made just after administration of benadryl.

On two occasions, once the ear temperature had again returned to preinjection levels, an H_2 receptor blocking agent, metiamide (N methyl N¹ (2 ((5 methylimidazole-4-yl) methylthio) ethyl) thiourea) (Metamide ^{R}, Smith, Kline and French Laboratories Ltd., Welwyn Garden City, Hertfordshire, England) was infused via the jugular catheter at 0.25 or 0.375 mg/kg for 10 min. This was in turn immediately followed by a further 500 µg subcutaneous dose of histamine-

HCl. Rectal temperature was recorded before, during and after each infusion.

The dilatory responses to histamine injections before and after treatment with histamine blocking agents were evaluated by determining the maximum ear surface temperatures of the injected as well as the opposite ear and also by determining the areas under the ear temperature curves, with a planimeter. The areas under the temperature curves were determined over 90 min following injection and used as the basis for calculating the percent inhibition of dilatation induced by benadryl.

The results of experiment 2-1 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keuls' test (Snedecor and Cochran 1967). The Student Newman-Keuls' test was applied using the harmonic mean number of observations per treatment.

Experiment 2-2: The Effect of Histamine Blocking Agents on the Hunting Response in the Ears of Cold Exposed Sheep

1. Experimental Animals

Experiment 2-2 consisted of six trials conducted on the same three adult wethers (two trials per sheep) as described in experiment 2-1. These trials examined the effect of histamine blocking agents on the "hunting response" in cold exposed sheep.

2. Catheterization

An indwelling Tygon catheter was installed in the jugular vein of the experimental animal as described for experiment 2-1. This catheter was prevented from freezing by the circulating water apparatus of Hills *et al.* (1977).

3. Temperature Recording

A 36 gauge thermocouple of the same type described in experiment 2-1 was attached to the mid-dorsal surface of each ear about 5 cm from the tip of the ear. These thermocouples were held in place in the same manner as described for experiment 2-1 and passed from the sheep via a port hole to a sampling and recording station located outside the temperature controlled room. Surface temperature and rectal temperature were recorded as described for experiment 2-1.

4. Heart Rate Recording

The electrocardiogram electrodes were installed as described for experiment 2-1.

5. Restraint of the Sheep During Trials

The animals stood in a metabolic cage as described in chapter 1. A companion wether stood in an identical metabolic cage in the temperature controlled room about 2 m to the left of the experimental animal.

6. Experimental Protocol

After a 30 min recovery period the room temperature was lowered to approximately -25C. After approximately 80 min, a "hunting response" was well established as indicated by the temperature recording of the dorsal surface of the ears. An infusion of 0.375 mg/kg of benadryl was then made over a 10 min period. In two of the trials, a single infusion of 0.2 mg/kg metamide was given over a 10 min period about 1 h after the benadryl. The heart rate and ear surface temperature were recorded before, during and after benadryl and metiamide infusions. For purposes of analysis in experiment 2-2 mean ear surface temperatures were calculated over 30 min periods before, during, and after each infusion. Mean heart rate was calculated over 5 min periods before and after each 10 min infusion of benadryl or metiamide. Ear surface temperature was then recorded for a further period up to approximately 110 min. At the end of this time the cooler was turned off and the room allowed to warm to the pre-experimental temperature.

The results of experiment 2-2 were analyzed statistically as described in experiment 2-1.

RESULTS AND DISCUSSION

Experiment 2-1

The data for preliminary trials in which compound 48/80 was infused via the carotid artery and histamine-HCl was infused via the carotid artery or the jugular vein are presented in Appendix 1. A consistent response of ear surface temperature was not obtained in these trials. It could not be determined whether the lack of consistent response to compound 48/80 and histamine-HCl in the peripheral vasculature was due to a lack of effect of these compounds on the peripheral vasculature or whether insufficient quantities were reaching the peripheral tissue via the constricted vasculature as suggested by Duff *et al.* (1953) for the histamine they infused into cold fingers. Therefore subcutaneous injections of histamine-HCl were used to insure a high local level of histamine in the cold constricted tissue.

Subcutaneous injection of 10 to 200 μg of histamine-HCl into cold exposed sheep produced no change in the surface temperature of the injected ears. On the other hand, injection of 500 μg of histamine-HCl always produced a large increase in the surface temperature of the injected ear as shown in Figs. 1 and 2, and Table 3. This increase was due to a local effect of the histamine-HCl on the peripheral vasculature as a similar increase in temperature was not

Figure 1

The effect of intravenous benadryl on the ear surface temperature elevated by subcutaneous injections of histamine-HCl

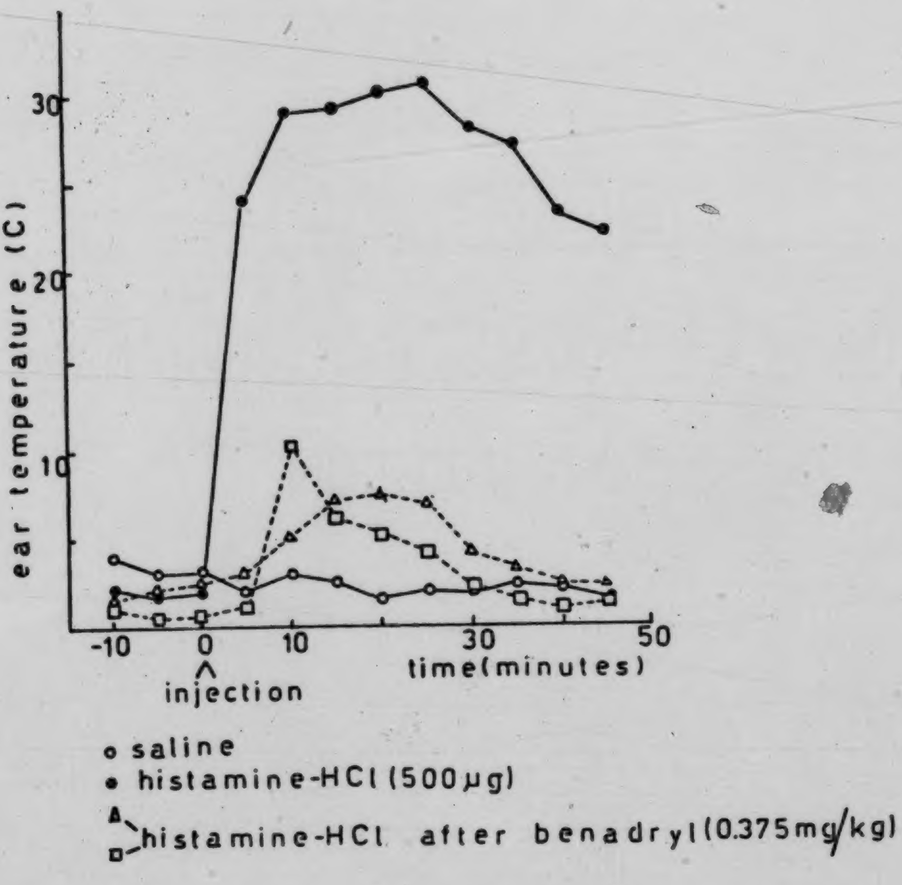


Figure 2

The effect of subcutaneous injection of physiological saline or histamine-HCl on ear surface temperature in sheep

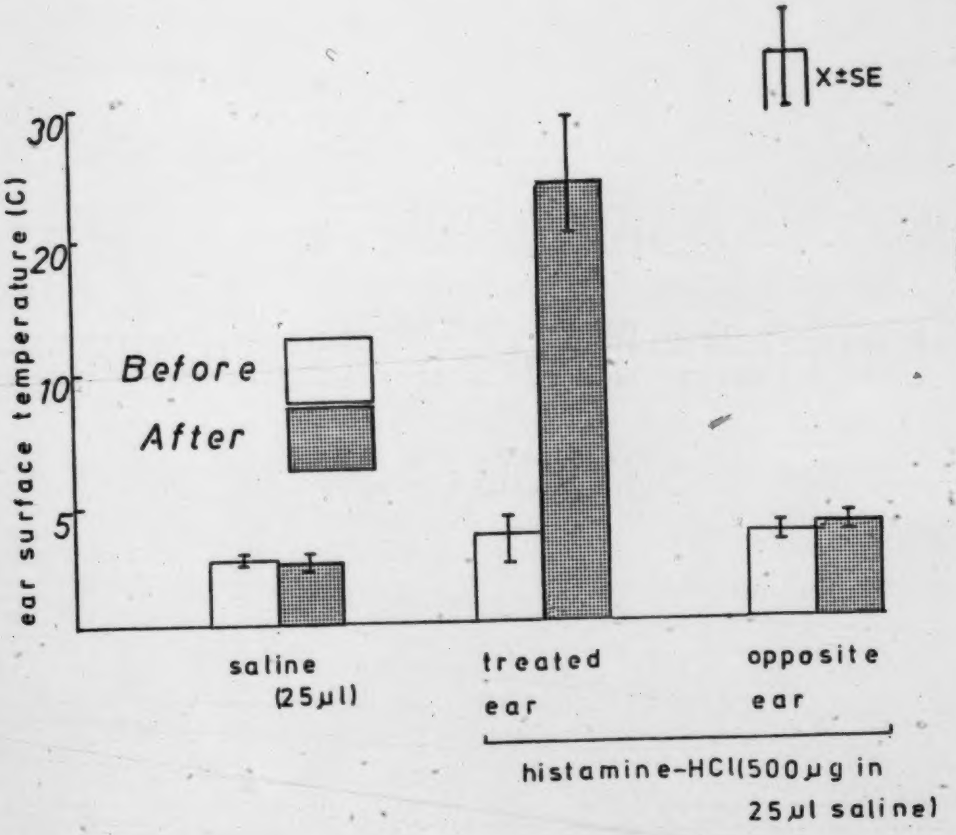


Table 3. The effect of a subcutaneous injection of 500 µg of histamine-HCl on the maximum ear skin temperature in cold exposed (1.5-2C) sheep. Experiment 2-1.

		Maximum Ear Temperature (C)			
		Pre Injection		Post Injection	
Trial	Animal No.	Injected ear	Other ear	Injected ear	Other ear
1	3	4.5	4.5	32.6	4.3
2	2	6.5	6.6	27.0	6.0
3	3	5.0	5.6	33.0	8.0
4	2	12.7	5.8	28.4	6.5
5	1	8.0	8.5	14.4	7.5
6	3	5.2	5.2	31.7	5.5
7	1	1.2	3.3	30.2	3.0
8	2	1.9	7.2	17.3	9.1
Mean		5.6^a	5.8^a	26.8^b	6.2^a
SE[1]		1.3	0.6	2.2	0.7

[1] SE-Standard error of the mean.

[a,b] -Means with different superscripts differ significantly ($P<0.05$).

found in the control ear of the sheep in response to injections of the same volume of physiological saline, Table 4. This latter result indicated that the minute volume of fluid injected did not cause sufficient trauma to induce a vascular response.

Subcutaneous injection of histamine caused slight transient increases of about 30 beats/min in heart rate immediately following injection. There was no effect of histamine on rectal temperature.

Table 5, and Fig. 1, show that the dilatory response to histamine could be reduced by previous jugular injection of 0.2 mg/kg or 0.375 mg/kg benadryl, an H_1 histamine receptor blocking agent. Larger doses (0.5 mg/kg) did not appear to have an inhibitory effect, or as in the case of 0.75 mg/kg may have slightly augmented the dilatory response (Fig. 3). These results suggest that histamine may be acting on more than one site. There is evidence for the presence of histamine receptors on sympathetic nerve endings as well as the vascular smooth muscle. McGrath and Shepherd (1976, 1978) were able to show that histamine depressed the contraction of dog saphenous vein strips in response to stimulation of their sympathetic nerves. The evidence presented for this response being due to a depression of the release of noradrenaline was as follows: (1) contractions caused by activating the nerve endings electrically or by

Table 4. The effects of a 26 µl of subcutaneous injection of physiological saline on the ear surface temperature in cold exposed (1.5-2C) sheep. Experiment 2-1.

Trial	Animal No.	Ear Skin Temperature (C)	
		Pre Saline	Post Saline
1	3	2.9	2.0
2	2	5.6	5.6
3	3	5.2	4.4
4	2	5.5	5.5
6	3	4.3	4.0
Mean		4.6	4.3
SE[1]		0.5	0.5

---

[1] SE-Standard error of the means.

Table 5. The percent inhibition caused by benadryl on the dilatory response of ear skin temperature following subcutaneous doses of 500 µg of histamine in cold exposed (1.5-2C) sheep. Experiment 2-1.

Benadryl dose (mg/kg)	Number of Observations	Percent inhibition due to benadryl	
		Mean	SE[1]
0.2	2	52	7.0
0.375	3	71.8	14.9
0.50	2	- 1.04	0.1
0.75	2	-17.0	1.5

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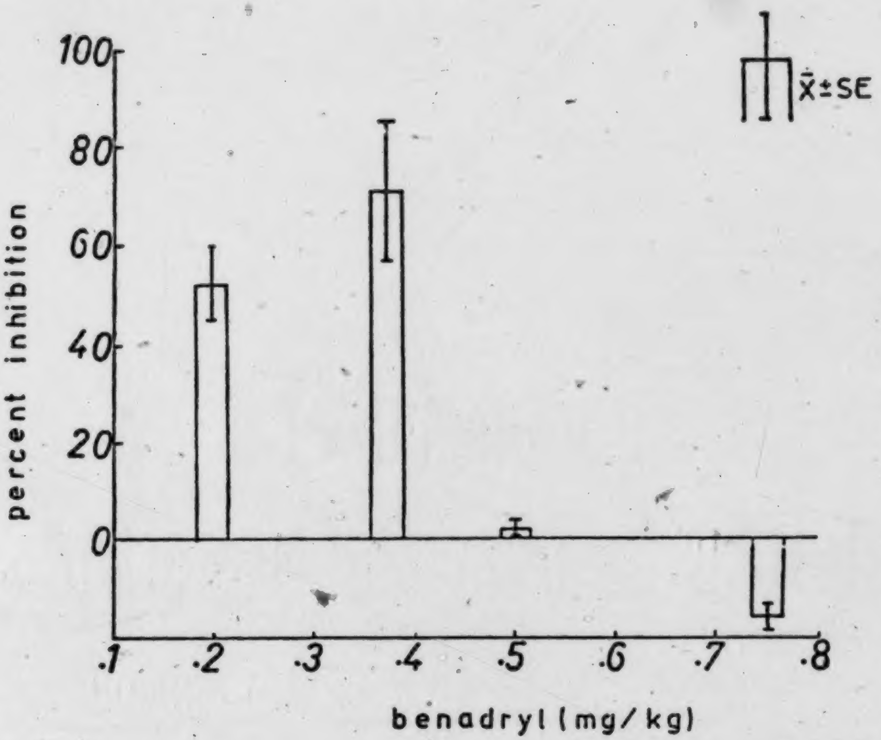
[1] SE-Standard error of the means.

Figure 3
The percent inhibition in the dilatory response in the ears of sheep to 500μg of subcutaneously injected histamine-HCl caused by increasing doses of benadryl



depolarizing with potassium ions were depressed by histamine, contractions caused by tyramine and noradrenaline were unchanged or augmented. (2) histamine depressed the release of (7-^{3}H) noradrenaline following electrical stimulation, whereas the release of radioactive compounds by tyramine were unaffected. They concluded that the receptor site was of the H_2 type as the H_2 blocker metamide inhibited the depression of the contraction response and the release of radioactive noradrenaline while the H_1 receptor antagonist pyrilamine did not. Also, the contractions due to electrical stimulation could be depressed by the H_2 agonist 4 methylhistamine and were augmented by the H_1 agonist 2 methyhistamine. Westfall (1977) considers that the potentiation of vascular smooth muscle contractions seen in dogs with higher levels of histamine are due to H_1 receptors on the smooth muscle cells.

The results of the present experiments suggest that, in sheep, there may be H_1 dilatory receptors with high affinity for histamine and H_1 constrictor receptors with low histamine affinity. The former may inhibit noradrenaline release from adrenergic nerve endings, while the latter may be located on the vascular smooth muscle cells where they act directly to cause constriction. Low doses of benadryl may have primarily blocked the former, while high doses may have blocked both groups of receptors. Westfall (1977) has indicated that sympathetic nerve transmission is depressed by lower concentrations of histamine than those required to stimulate smooth muscle directly. This suggests that histamine receptors on adrenergic nerves may have a higher affinity for histamine (and presumably blocking agents) than those on the smooth muscles. In the presence of higher doses of benadryl, the dilatory effects of histamine may have been caused by activation of H_2 dilatory receptors. H_2 effects are often masked by the responses of H_1 receptors, but become apparent after H_2 receptors are blocked. This was reported by Glover et al. (1973) in the ears of rabbits in which the vasoconstriction in response to histamine became a vasodilatation in the presence of an H_1 blocker. The dilatation could be blocked by an H_2 blocker.

On the two occasions when the H_2 receptor blocker metiamide was given following the H_1 blocker benadryl, there was no further change in the response of the ear skin temperature to subcutaneous histamine injection. While this observation casts some doubt on the dilatory role of H_2 receptors, the dose rate of metiamide may not have been sufficient to block the receptor sites.

In other species both H_1 and H_2 types of histamine receptors have been found to participate in the vascular responses to histamine. In the isolated rabbit ear, Ercan and Tucker (1975) found both H_1 and H_2 receptors for histamine. In this case activation of H_1 receptors appeared to cause a constriction of the vessels while activation of H_2 receptors appeared to cause a dilatation of the vessels after H_1 blockade. The present results indicate that the dilatation of the vasculature of the ear in cold exposed sheep in response to exogenous histamine may be mediated by H_1 receptor sites. Other responses in sheep have been found to be mediated by H_1 receptor sites, without the participation of H_2 receptor sites. Brigham *et al.* (1976) were able to completely block the edema in the lungs of sheep in response to histamine with the H_1 blocker diphenhydramine. The dilatation of the blood vessels in the sheep ear being an H_1 mediated response is in agreement with the results of Eyre and Wells (1973) in calves where H_1 receptors appear to give a depressor response. In calves however, activation of H_2 receptors apparently produces a pressor (constrictor) response. Further experiments are needed to clearly define the role of H_2 receptors in the peripheral circulation in sheep.

Experiment 2 - 2

The results of experiment 2 - 1 indicated that cold constricted ears of sheep are capable of dilating in response to exogenous locally applied histamine and that this response can be reduced by the use of an H_1 histamine receptor blocking agent. In experiment 2 - 2 jugular infusions of 0.375 mg/kg benadryl (the dose rate from experiment 2 - 1 which produced the maximum inhibition of the dilatory response to exogenous histamine HCl) into cold exposed (-25C) sheep did not significantly affect maximum, minimum or maximum minus minimum ear skin temperature or the interval between "hunting responses" as shown in Fig. 4 and Table 6. There was no significant increase in heart rate following benadryl (Fig. 5). Treatment with 0.25 mg/kg metiamide did not influence skin temperature. These results indicate that the naturally occurring "hunting response" in the ears of sheep was not affected by jugular infusions of the H_1 blocking agent or a subsequent dose of an H_2 blocking agent. These results are in agreement with the results obtained by Whelan (1956) for the human index finger. He found that benadryl infused at 1.5 mg/min did not produce a difference in the heat elimination records between cold (0-6C) exposed index fingers and control fingers recorded 30 min before treatment. These results indicate that the action of histamine on H_1 receptors is not the mechechanism

Figure 4

The effect of benadryl on maximum and minimum ear skin temperature in cold exposed (-25C) sheep

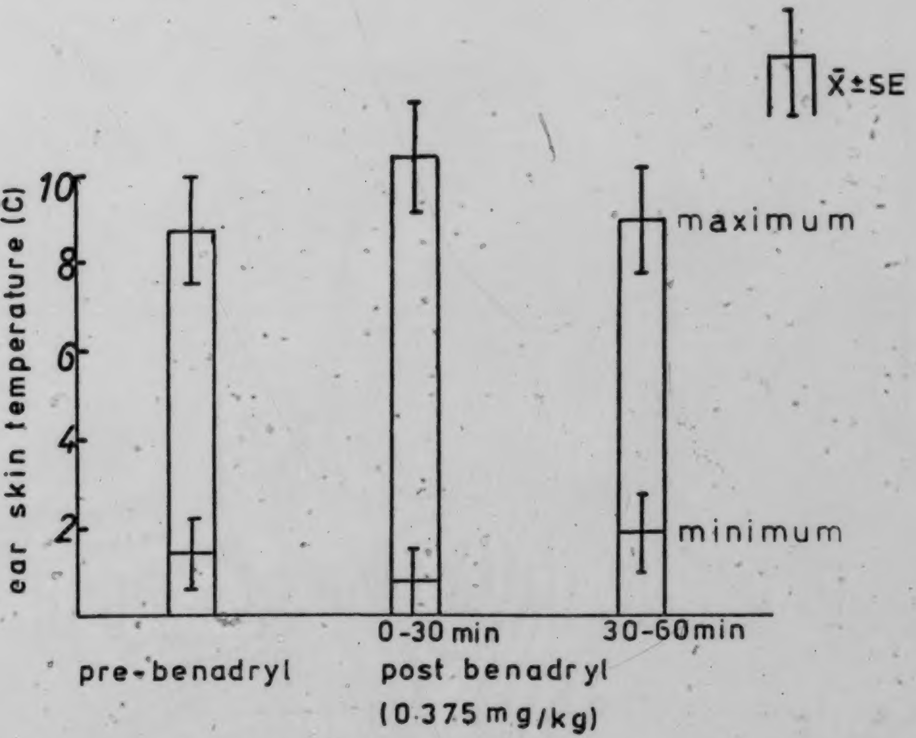


Table 6. The effect of benadryl on mean maximum, minimum and maximum minus minimum ear skin temperatures, interval between "hunting responses" and heart rate in cold exposed (-25C) sheep. Experiment 2-2.

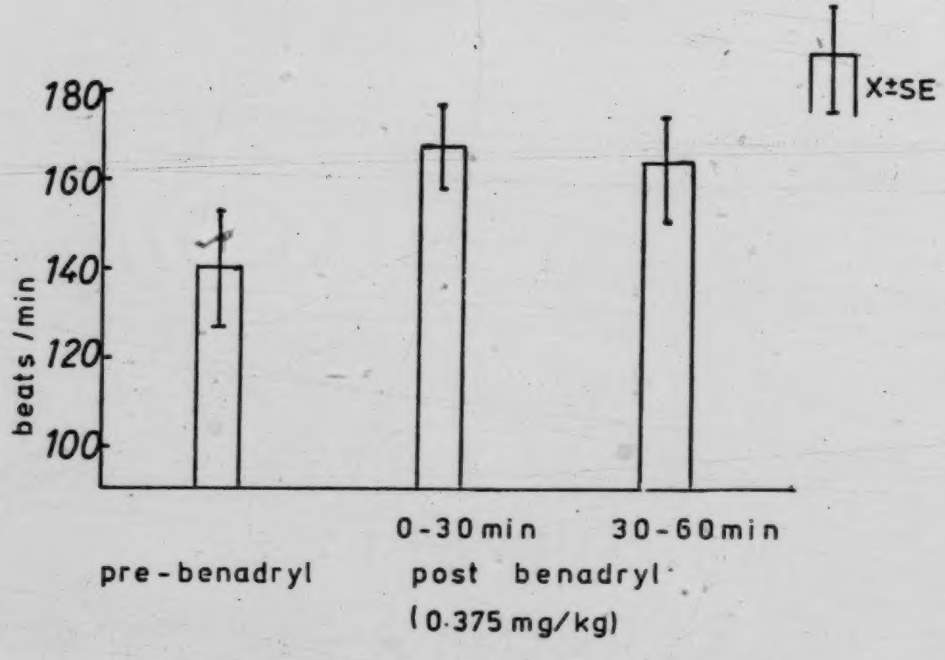
	Ear Skin Temperature (C)				
	Maximum	Minimum	Maximum minus minimum	Interval (s)	Heart rate (beat/min)
Pre benadryl[1] (30 min)	8.71(18)[2]	1.40(18)	7.31	408.1(15)	139.0(18)
Post benadryl (0-30 min)	10.40(18)	0.82(18)	9.57	404.2(15)	167.8(18)
Post benadryl (30-60 min)	8.95(16)	1.96(16)	7.00	375.7(13)	162.8(18)
SE[3]	1.18	0.80	1.12	33.4	11.13

[1] Benadryl was infused at 0.375 mg/kg body weight.

[2] Number of observations per mean. Each mean is the result of repeated observations on each of three sheep.

[3] SE-Standard error of the mean.

Figure 5
The effect of benadryl on heart rate in cold exposed (-25C) sheep



for the "hunting response" in the ears of sheep. While the results cast some doubt on the possible role of H_2 receptors, their participation in cold induced vasodilatation cannot be ruled out on the basis of these experiments. Further experiments are needed to clearly define the role of H_2 receptors in the peripheral circulation of sheep.

Chapter 3 - The Role of the Sympathetic Nervous System in Vasoconstriction and Cold Induced Vasodilatation.

LITERATURE REVIEW

In environments in which the temperature extends below 0C exposed extremities will freeze if additional heat is not supplied as demonstrated in finger skin by Wilson et al. (1976). This increased heat must be supplied by warm blood from the interior of the animal as the peripheral tissues, particularly those in tails and ears which do not have large underlying masses of muscle, have limited metabolic capacity. Thus periodic increases in blood flow to the extremities exposed to freezing temperatures are required to prevent damage to the tissue from freezing yet preventing excessive heat loss to the environment that would result from continuous blood flow. This periodic increasing and decreasing of extremity temperature was first described by Lewis in 1930 as the "hunting phenomenon" but now commonly referred to as the "hunting response". The control of the "hunting response" is not understood.

Considerable evidence for the constriction of the skin vasculature in response to noradrenaline, the neurotransmitter substance released at sympathetic nerve endings, has been presented by Shepherd and Vanhoutte (1975). This constriction is probably mediated by α receptors on the vascular smooth muscle as defined by

Ahlquist (1948). Moser et al. (1953) were able to show that the α blocking agent dibenzyline produced an increase in blood flow of two to six fold in the lower extremeties of individuals given the drug in a 22 to 25C environment suggesting blocking of an α receptor mediated constriction of the peripheral circulation.

In a cool or cold environment the reduction in blood flow to the extremities has been attributed to the action of the sympathetic nerves by Donegon (1921). Heitman (1972) reported that the α blocker phenoybenzamine increased skin temperature in cold exposed sheep (-17C). Meyer and Webster (1971) abolished the constriction of the blood vessels of the ear on one side of a cold exposed sheep by surgical sympathectomy of that side of the head.

Two observations support the concept that the "hunting response" is controlled by a local mechanism rather than by a centrally controlled neural or hormonal mechanism. First, Greenfield et al. (1951a, 1952) found that in subjects with complete nerve section and subsequent degeneration cold vasodilatation could still be obtained although the response was greatly modified. The degree of dilatation being usually less in the denervated finger and gradually increased as "regeneration" occurred. They suggested from these studies that a neural mechanism was not responsible for the "hunting response". Second, studies on the human finger (Hertzman and Roth 1941) and the ear of the domestic sheep (Meyer and Webster 1971) have demonstrated that the increase in blood flow to any two of these extremities in the cold occurred asynchronously, again suggesting a local control of the blood flow within an extremity.

Many factors have been proposed as the local dilatory agent in the peripheral circulatory system. Lewis (1927) originally proposed histamine as a dilator in the vascular system. On the other hand, Webster (1974a) suggested that histamine was an unlikely candidate for the proposed dilator substance in the cold. He proposed instead a vasoactive polypeptide as described by Hilton (1962). The work of Armstrong and Mills (1965) is in agreement with this suggestion as they found that decreasing the temperature at which plasma is stored from 37 to 0C caused a depletion of kininogen with a transient presence of kinin. Mashford and Zacest (1967) however, found a fall in the bradykinin concentration in venous flow from the hand in response to increased sympathetic activity during cold exposure (0C). This suggested to them that bradykinin was not a mediator of cold induced vasodilatation. Schayer (1970) suggested that polypeptides do not meet the required criteria of a local dilator. He considers the criteria to be (a) it must be formed "intrinsically", that is, its protein precursor must be found intra-cellularly, not in the plasma, (b) it must be formed by an inducible enzyme system, (c) drugs which destroy it or block its activities must alter microcirculatory control, (d) it must not show signs of tachyphylaxis. He again suggested that histamine, formed by what he called an "inducible enzyme" might well serve as a local vasodilatory agent. The results of experiments described in chapter two of this thesis, however, suggest that H1 histamine receptors which can mediate vasodilatation in sheep do not play a role in the "hunting response".

Meyer and Webster (1971) claimed that the withdrawal of sympathetic stimulation was not the cause of cold induced vasodilatation as they were able to demonstrate the "hunting phenomenon" in the sympathectomized ear of a sheep during the continuous infusion of noradrenaline. However, it can also be seen from their data that no "hunting" took place concurrently in the opposite intact ear. This data may also be interpreted as showing that on the intact side, sufficient noradrenaline was provided by the sympathetic nervous system together with the infusion to maintain a consistent constriction. However, on the sympathectomized side, all the noradrenaline would be expected to enter with the blood flow. As the constriction took place and blood flow declined, the amount of noradrenaline reaching the receptor sites would tend to decline until insufficient was present to maintain the constriction. This might result in a passive dilatation, an increase in blood flow, an influx of more exogenous noradrenaline, and a reinitiation of the constrictor phase of the cycle.

Other possible explanations for cold induced vasodilatation include the failure of noradrenaline release from sympathetic nerves in the cool tissue. Shepherd and Vanhoutte (1975) reported that local cooling to 28C depressed the release of ^{3}H-noradrenaline from the isolated saphenous vein of the dog during electrical stimulation. However, the contraction of the vessel was augmented by cooling (Vanhoutte and Shepherd 1970). A similar phenomenon has been reported in the intact anesthetized dog by Webb-Peploe and Shepherd (1968) and Abdel-Sayed _et al._ (1970). Although Thompson _et al._ (1978) reported an increase in the noradrenaline content of plasma draining the head of cold exposed sheep, the contribution of the peripheral tissue to this increase was not determined.

The constriction response of cooled vessels to sympathetic stimulation appears to be a property of vascular smooth muscle rather than a property of superficial veins alone. Vanhoutte and Lorenz (1970) found that isolated saphenous and mesenteric veins when cooled increased constriction in response to electrical stimulation noradrenaline, and 5 hydroxytryptamine. Contraction of the cooled femoral vein however was depressed in response to these same stimulations. Abdel-Sayed *et al.* (1970) and Absolon *et al.* (1971) reported the portal vein in intact animals constricted, but the deeper limb veins dilated when cooled. This dilatation of the deeper limb veins on cooling would facilitate the shift of blood from the constricted surface vessels to the deeper veins as a heat conservation mechanism.

Keatinge (1958) found that tissue temperatures lower than 10C result in a failure of isolated vascular smooth muscle strips to constrict in response to adrenaline. The ability of peripheral veins to continue to constrict in response to noradrenaline even at very low temperatures (2C) has, however, been demonstrated by Millard and Reite (1975) in the excised legs of domestic ducks (*Anas boscas*). Meyer and Webster (1971) maintained continuous constriction in the intact ear of a cold exposed sheep by jugular infusion of noradrenaline.

The constriction of peripheral blood vessels in response to cold causes a large reduction in the flow of blood to the cold exposed tissue. Burton and Edholm (1955) found a reduction from 3 ml/100 ml of forearm/min to 0.75 ml/100 ml of forearm/min when an arm was transferred from a 37C bath to a 15C bath. Greenberg (1963) reported that a flow of greater than 30 ml/100 ml of toe/min at a room temperature of 43C was reduced to only 3 ml/100 ml of toe/min in a room at 20.5C. This lower flow might reduce the amount of oxygen available to the tissue and permit a build up of metabolic end products within the tissue. Either oxygen lack or build up of metabolic end products might cause the vascular smooth muscle to relax. Vanhoutte (1973) has shown that anoxia will reduce the ability of veins to constrict in response to noradrenaline. This response was more marked in the mesenteric veins than in the saphenous vein. In the absence of glucose, the depression of contraction due to lack of oxygen was equal in both veins. This suggested to Vanhoutte and several co-authors (Vanhoutte 1970, Vanhoutte and Leusen 1972, Shepherd and Vanhoutte 1975) that the subcutaneous veins were more capable of switching to anaerobic metabolism to maintain the contractile process.

The reaction of veins to nerve stimulation has been shown to be more susceptible to the depressing effects of anoxia than the reaction of veins to exogenous noradrenaline. This has suggested to several authors (Vanhoutte 1970, Paton 1972, Vanhoutte and Leusen 1972, and Kirpekar et al. 1973) that anoxia decreases the rate of release of the transmitter. In spite of these responses in isolated vessels, Shepherd and Vanhoutte (1975) state "Severe oxygen deprivation and substrate depletion must be combined before the reactivity of the venous smooth muscle is markedly altered". These authors imply that in the intact organism, local variations in oxygen are not important regulators of the venous system. Thus, the reactivity of the veins is unaltered by the usual fluctuations in oxygen content. Therefore, if anoxia is not likely the cause of cold induced vasodilatation, then yet another mechanism must be sought to account for the phenomenon in the sympathetically constricted extemities. Changes in hydrogen ion and carbon dioxide concentrations are known to affect both venous smooth muscle contraction and the response of smooth muscle to sympathetic stimulation. A decrease of pH from 7.4 to 6.8 caused a relaxation an increase to pH 7.8 augmented myogenic activity and basal tension in the mesenteric veins (Vanhoutte 1967). Similar changes in pH in cutaneous veins did not affect resting tension. When pH was increased from 6.8 to 7.4 by altering bicarbonate concentration, the constrictor responses of both mesenteric and cutaneous veins to nerve stimulation progressively increased. Since pH change caused a non-parallel shift of the Ca^{2+} effect on nerve stimulation (Vanhoutte 1968), it was suggested that the pH changes influenced both the adrenergic nerve ending and the smooth muscle. Decreasing pH from 7.8 to 6.8 by increasing pCO_2 depressed the constrictor response of mesenteric and cutaneous veins to nerve stimulation, while decreased pCO_2 had an opposite effect. For the same decrease in pH the constrictor response was more depressed by a decrease in bicarbonate than by an increased CO_2 (Vanhoutte and Clement 1968). If pCO_2 was increased at a constant pH the constrictor response of isolated veins to nerve stimulation was augmented (Vanhoutte and Clement 1968). Increasing pCO_2 did not, however, enhance the constrictor response to exogenous l-adrenaline or l-noradrenaline. This was cited by Shepherd and Vanhoutte (1975) as evidence that CO_2 might cause a facilitation of neurotransmitter release.

Although the venous bed is exposed to the metabolic changes that occur in tissues, the periphery of an animal is not considered to have a high metabolic capacity. This small metabolic activity may be further reduced by the cooling of the tissue in a cold environment. Thus changes in metabolic parameters in peripheral tissues might be so slight as to be unimportant in the control of blood flow to the extremeties. If it does occur, autoregulation of blood flow by metabolites would be expected to influence capillary flow in small localized regions of time and probably would not result in large syncronized fluctuations typical of the hunting response.

In this experiment the increased flow observed during the hunting response might have been through arterio-venous anastomoses, capillaries, or both. The first observation of artio-venious anastomasis *in vivo* was in the ear of the rabbit by Grant in (1930). Arterio-venous anastomosis were later identified in sheep skin by Daniel and Prichard (1956), and Molyneaux 1965).

In thermoneutral environments blood flow through the arterio-venous anastomoses has been considered to be regulated by α receptors alone. Spence *et al.* (1972) used microspheres to demonstrate an increased blood flow through the aterio-venous anastomses in the skin of the hindlimb of the dog, after injection of l-noradrenaline and a reduction in the response following the α blocking agent phentolamine. The β stimulating agent isoproterenol increased capillary flow and the β blocking agent propranolol hydrochloride reduced capillary flow, but these agents had no effect on flow through the arterio-venous anastomses. Webster (1974a), on the other hand, states: "capillary flow in the superficial tissues is maintained by both active vasoconstrictor and vasodilator sympathetic stimuli acting through a and β receptors respectively ". However, Wiedman *et al.* (1976) in discussing the precapillary sphincter states a third possibility: "Unless refuted by further anatomical studies it" (the precapillary sphincter) "could be recognized as having contractile activity of a myogenic nature and being completely devoid of direct control by the autonomic nervous system." Thus , at the capillary level, sufficient blood may pass through the constricted vessels to maintain the comparatively small metabolic needs of the peripheral tissue. This may be the mechanism described as "continuous proportional control" (Meyers and Webster 1971). The thermal needs of this tissue could be maintained by a separate mechanism perhaps involving control of the artio-venous anatomses by the "hunting response".

It is also possible that cold induced vasodilatations involve increased capillary flow. There is recent evidence presented by Fregly et al. (1976) that β adrenergic agonists (isoproterenol and adrenaline) can cause vasodilatation in the cold exposed tail of the rat. This dilatation could be blocked by the β blocking agent propranolal. Therefore there remains the possibility that the "hunting response" could be caused by activation of β receptors in vascular smooth muscle. In view of the work of Spence et al. (1972) the possibility exists that this flow increase may be partially due to increased capillary flow. As noted previously individual extemities have been noted to "hunt" independently of each other. However the degree of independence is still a matter of contention. Whittow (1962) reported that the temperature of the ears of calves "usually increased and decreased together.." while Meyer and Webster (1971) reported that several authors (Lewis 1930, Greenfield et al. 1951b and Webster and

Blaxter 1966) suggest that the "hunting phenomenon" does not take place synchronously in all extemities. It has not been established whether different surface sites of the same extremity "hunt" synchronously. Therefore, an experiment was conducted to clarify this point. The remaining series of experiments were designed to further explore the relationship of the sympathetic nervous system to the constriction and dilatation of peripheral blood vessels in the ears of cold exposed sheep. In particular the changes in catecholamine outflow from a sympathectomized portion of the head, the possible role of β receptors and the ability of the extremities to dilate under continuous sympathetic neural stimulation were investigated.

MATERIALS AND METHODS

Experiment 3-1: Determination of the area of independence cf a "hunting response".

The extremities of domestic animals faced with an acute cold exposure have been shown to dilate and constrict independently of each other (Meyer and Webster 1971). In order to determine if different areas of skin within a given extemity dilate and constrict synchroncusly or asychronously, the four trials of experiment 3-1 were performed.

1. Experimental Animals

Experiment 3-1 was conducted using one Suffolk and two Cheviot adult wether sheep (mean weight 87.3 ± 8 kg). The sheep were maintained in an indoor pen at a room temperature of approximately 20C. The sheep consumed 1000 g of dehydrated alfalfa pellets per day divided into two equal portions fed at approximately 0800 hours and 1600 hours daily. The second portion of the feed was fed at the end of the trial if the trial extended later than 1600 hours. On the day of a trial the experimental animal was not fed the 0800 hour portion of feed and preparation of the arimal for the experiment began at this time.

2. Temperature Recording

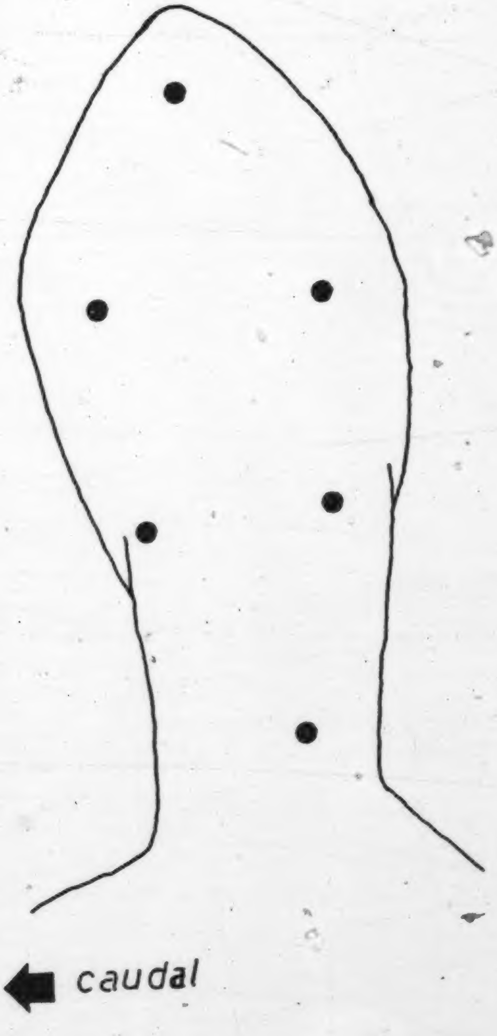
On the day of a trial, the experimental animal and a companion animal were placed in individual metabolic cages in a temperature controlled room as previously described. Six 36 gauge thermocouples were distributed on the dorsal surface of the right ear, 4 to 5 cm apart, as shown in Figure 6. Two identical thermocouples were fastened to the opposite ear. All thermocouples were connected to a Honeywell model 15 multipoint temperature recorder which had been wired in parallel to read these eight thermocouples in rotation such that 45 s elapsed between successive readings of the same point. A rectal thermistor was inserted into the animal as previously described in chapter 2 to record rectal temperature.

3. Experimental Protocol

After a 30 min waiting period, at 20.5C, the cooler which had been previously set to produce its maximum cooling output was turned on and the room was cooled. The animals ear surface temperature and rectal temperature were recorded for approximately 190 min after the cooler was turned on. At the end of this time period the cooler was turned off and the room allowed to warm to the pre-experimental temperature.

Figure 6

The position of the six thermocouples on the right ear



Experiment 3-2: Effects of acute cold exposure on arterial and venous concentrations of adrenaline and noradrenaline in sheep.

One of the possible mechanisms responsible for cold induced vasodilatation is a reduction in the extent of sympathetically induced vasoconstrictor tone in peripheral tissues. Changes in sympathetically induced vasoconstriction could be caused by an alteration in output or uptake of noradrenaline by the peripheral nerves or by a change in the amount of enzymatic breakdown of noradrenaline taking place in the cold peripheral tissue.

1. Experimental Animals

In order to determine if arterio-venous differences in concentration of noradrenaline across the head are related to exposure temperature, four trials were carried out on the three adult wethers housed and fed as described in experiment 3-1.

2. Catheterization

On the day of a trial the right jugular vein and the right carotid artery of the experimental animal were catheterized as previously described in chapter 2 for the jugular vein. The carotid catheter was inserted via a carotid loop which had been surgically installed as described by Hecker (1974). Both catheters were protected from freezing as described by Hills *et al.* (1977).

3. Experimental Protocol

After preparation, the experimental animal was left undisturbed at a temperature of 23.7 ± 0.4C for 1 h. At the end of this 1 h waiting period, heart rate had stabilized and the animal was standing quietly in its metabolic cage. Blood samples (20 ml) were then simultaneously withdrawn from both the jugular vein and the carotid artery. A second set of simultaneous samples was withdrawn 30 min later, following which the cooler which had been previously set to produce its maximum cooling capacity was turned on.

The animal's environment was cooled for 1 h at which time the room temperature had reached -20.3 ± 0.7C. At this time, a third set of simultaneous blood samples was taken. Two further sets of samples were taken 30 and 60 min later. After the third blood sample was collected in the cold, the cooler was turned off and the animals envircnment warmed to a minimum of 10C over a period of 90 min when the final set of blood samples was taken. All samples were collected and analyzed for plasma adrenaline and noradrenaline concentrations as described by Thompson *et al.* (1978).

4. Statistical Analysis

The results of experiment 3-2 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keuls' test (Snedecor and Cochrane 1967).

6

Experiment 3-3: Effect of cold exposure on concentrations of adrenaline and noradrenaline in plasma returning from sympathectomized and non sympathectomized sides of the head.

In order to assess the contribution of peripheral sympathetic nerves to the concentration of plasma noradrenaline observed in acutely cold exposed animals, two trials were carried out.

1. Experimental Animals

A crossbred Suffolk x Chevoit wether weighing 48 kg was surgically prepared by removal of about 2 cm of the right vago-sympathetic trunk in the mid cervical region. The surgery was performed with the animal under general gaseous anesthesia (Halothane, Fluothane ^{R} Ayerst Laboratories Ltd., Montreal, Quebec) following sterile surgical technique. The animal was allowed 4 d to recover from the surgery prior to use in experiments.

2. Catheterization

On the day of a trial the sheep was prepared by the insertion of two Tygon ^{R} catheters (1.0 mm inner diameter and 1.8 mm outer diameter), 15 cm into the right and left jugular veins by the use of a 12 gauge needle. The exterior 3.7 m of these catheters were then enclosed in the apparatus of Hills *et al.* (1977) to prevent freezing.

Electrodes to record heart rate, rectal temperature probe and ear skin thermocouples were installed as described in chapter 2. The animal was placed in a metabolic cage in a temperature controlled room in the company of another sheep in an identical metabolic cage, 2 m to the left of the experimental animal.

3. Experimental Protocol

After 30 min the heart rate had stabilized and blood samples (20 ml) were collected simultaneously from each of the jugular catheters. During each trial, blood samples were collected at 0 and 30 min prior to cooling the room. The cooler, which had been set to produce its maximum cooling capacity, was then turned on and the animals' environment allowed to cool for 60 min when a second set of samples was taken. Samples were subsequently taken at 90 and 120 min. The cooler was then turned off and the environment allowed to warm for 30 min when a final set of samples was taken.

The samples were collected and analyzed for plasma adrenaline and noradrenaline as described by Thompson *et al.* (1978).

4. Statistical Analysis

The results of experiment 3-3 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keul's test (Snedecor and Cochran 1967).

Experiment 3-4: Determination of the concentration of adrenaline and noradrenaline in plasma samples collected during the ascending and descending temperature phases of the "hunting response"

If variations in the noradrenaline outflow from the sympathetic nerve endings are responsible for the "hunting response" a change in the overflow of noradrenaline into the blood draining from the peripheral tissue might be observable. In experiment 3-4 two trials were carried out to determine if plasma catecholamine concentrations differed in samples collected during the constriction and dilatation phases of individual "hunting responses".

1. Experimental Animals

Two adult Suffolk wethers weighing 73 and 76 kg were housed and prepared for experimentation as described in chapter 2, experiment 2-2. A jugular vein catheter was installed as described in chapter 2, experiment 2-1.

2. Experimental Protocol

At the end of a 30 min waiting period, the animals were standing quietly in the metabolic cages and the cooler which had been previously set to produce its maximum cooling capacity was turned on. The animals' environment was cooled until a "hunting response" was established as indicated by the temperature record of the dorsal surface of the ear on the same side of the body that the jugular catheter had been installed. Blood samples (20 ml) were withdrawn during the ascending and during the descending phases of individual "hunting responses". This proved difficult in every case due to the short time of ascent and descent of individual "hunts" and the relatively long period of time required to collect the large blood samples required for catecholamine analysis. Samples were obtained during both the ascending and descending phases of two "hunts" in the first trial and during five "hunts" in the second trial. These samples were prepared and analyzed for plasma adrenaline and noradrenaline concentrations as described by Thompson *et al.* (1978).

3. Statistical Analysis

The results of experiment 3-4 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keuls' test (Snedecor and Cochran 1967).

Experiment 3 - 5: Determination of the ability of the cold exposed ear of a sheep to hunt during continuous sympathetic stimulation

If the hunting response is due to an active dilator substance or the failure of the cold peripheral vasculature to respond to noradrenaline "hunting" might take place during the continuous sympathetic stimulation to constrict. Experiment 3-5 was designed to determine if cold induced vasodilatation could take place while noradrenaline was being released from the peripheral sympathetic nerve endings.

1. Experimental Animals

Seven trials were performed in which the *Vagus et Truncus Sympathicus* nerve was cut and stimulated in mature sheep anesthetized with halothane. Culled ewes of various breeding from the University of Alberta flock were used.

2. Surgical Preparation of the Animal

Each sheep was anesthetized and placed on its right side on a mobile operating table. A longtitudinal incision was made in the neck region about 5 cm to the left of the mid line and about 10 cm long. The left vago-sympathetic trunk was exposed and gently freed for about 5 to 7 cm from the carotid artery. A 60 cm long catheter (1.0 mm internal diameter and 1.8 mm external diameter) was surgically implanted 10 cm into the exposed carotid artery in each sheep. The retraction on the incision was then relaxed to allow the overlying muscle layers to cover the nerve and the carotid artery. The incision was covered with several gauze sponges moistened in physiological saline.

In the last three trials, arterial and auricular vein catheters were implanted. A second skin incision was made slightly below and behind the animals left ear. A catheter (1.0 mm inner diameter and 1.8 mm outer diameter) was surgically implanted 2 to 3 cm into one of the branches of the posterior auricular vein. The retraction on this incision was relaxed and it was similarly covered with saline soaked gauze sponges. A 36 gauge thermocouple was fastened to the mid-dorsal surface of the ear as described previously.

The animal was then gently rolled over onto its left side and moved so that its head extended over the end of the operating table. The head was suspended by a rubber sling so that the left ear hung downward and the area surrounding the ear was not compressed by the sling. By lowering the sling about 15 cm, the left ear could be immersed in a cooling bath maintained at 0C by means of ice water or at 0 ± 2C by means of a mixture of ethylene glycol and water cooled by an immersible refrigeration coil.

3. Experimental Protocol

Ear surface temperature was monitored continuously before and during immersion of the ear in the cooling bath. The incision in the animals neck was then retracted and the vago-sympathetic trunk was tied and severed. The peripheral segment of the nerve was gently placed across a pair of electrodes which had been prepared from foreshortened hair clips, from which the laquered surface had been removed. These electrodes were in turn fastened inside the barrel of a 10 ml plastic disposable syringe which had one side cut away. This electrode apparatus had the advantage of being able to hold the nerve with little obvious mechanical trauma while isolating the stimulating electrodes from the surrounding tissue. The nerve was given multiple 10 to 15 V stimulations at frequencies of 4 to 5 s^{-1} by means of a Harvard Model 340 Stimulator (Harvard Apparatus Company, Dover Massachusetts).

In the last three trials, blood samples were taken for determination of plasma catecholamines from the carotid artery and posterior auricular vein before and after immersion of the ear and after nerve severance and stimulation. The catecholamines were determined as described by Thompson *et al.* (1978). After collection of the last blood sample, the animals were killed by injecting 50 to 100 ml saturated magnesium sulphate into the carotid artery via the implanted catheter or a hypodermic needle.

Experiment 3-6: The effect of β-adrenergic agonists and antagonists on peripheral circulation in the cold.

The possibility exists that the "hunting response" is caused by activation of β-adrenergic dilator receptors in the peripheral vasculature. Experiment 3-6 was conducted to examine whether β agonists and antagonists effect the hunting response in the ears of cold exposed sheep.

1. Experimental Animals

Three trials were conducted on the three adult wethers described in experiment 3-1 to evaluate the participation of β-adrenergic receptors in the peripheral vasculature of sheep during acute cold exposure. On the day of a trial the right jugular vein of the experimental animal was catheterized as described in chapter 2 and the recording apparatus was installed as described in chapter 2, experiment 2-2.

2. Experimental Protocol

After a 30 min pre-experimental period, the heart rate had stabilized and the animal was standing quietly in its metabolic cage. The cooler, which had been previously set to produce its maximum cooling capacity, was turned on and the animals' environment cooled for 170 min.

A "hunting response" was established as indicated by fluctuations in the temperature record of the dorsal surface of the ears. At this time, 0.2 µg/kg isoproterenol (Isoprel^{R}, isoproterenol HCl, Winthrop Laboratories, Division of Sterling Drugs Ltd., Aurora, Ontario) from a solution containing 10 µg/ml was injected into the jugular vein. Approximately 25 min later an injection of 0.5 mg/kg of propranolol (propranolol-HCl, 2 mg/ml, Ayerst, McKenna and Harrison Ltd., Montreal, Quebec) was given. This was in turn followed by a second injection of isoproterenol (0.2 µg/kg) approximately 16 min later.

In the first trial three further injections of isoproterenol were given to test the duration of the β-receptor block by propranolol. These were given 45, 68 and 103 min following the propranolol. In the second trial no further isoproterenol was given. In the third trial two further injections of isoproterenol were given at 30 and 45 min after the propranolol dose. The response due to the second and subsequent doses of isoproterenol were pooled during the analysis of the results.

The temperature of the dorsal surface of the ears was recorded throughout these experiments using a Honeywell model 15 multipoint temperature recorder. Heart rate, rectal temperature, and room temperature were recorded before, during and after the injection of both drugs.

Experiment 3 - 7: The effects of subcutaneous isoproterenol on the ear skin temperature of cold exposed sheep

Experiment 3-7 was designed to investigate whether introducing the β-agonist isoproterenol subcutaneously into the ear would induce vasodilatation in the ears of cold exposed sheep. The subcutaneous route of administration was used to ensure a high concentration of the β-agonist at the ear skin vasculature.

1. Experimental Animals

Trials were carried out using the same three mature wethers, housed and fed as described in chapter 3-1. Each animal was prepared for experimentation by inserting a catheter containing 4 mg/ml isoproterenol physiological saline under the dorsal surface of the left ear. The catheters were identical to that used in the subcutaneous injections of histamine-HCl. A 36 gauge thermocouple was placed on the skin surface directly over the tip of the subcutaneous needle as described in chapter 2 for experiment 2-1.

2. Experimental Protocol

All three animals were placed in metabolic cages in a temperature controlled room as described in chapter 1. At the end of the 30 min waiting period, the room was cooled to

3 ± 2C, which was a sufficient degree of cooling to cause the peripheral vasculature of the animals to vasoconstrict.

Surface temperature and heart rate were monitored by the technique described in chapter 2 for 10 min before and for 10 min after each animal was given 100, 200 or 400 µg subcutaneous doses of isoproterenol solution via subcutaneous catheters. One animal destroyed the thermocouple to its ear before sufficient time had elapsed to take a surface temperature reading. The other two sheep received two injections of the 400 µg dose.

3. Statistical Analysis

The results of experiment 3-7 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keuls' test (Snedecor and Cochran 1967).

Experiment 3 - 8: The effects of intravenous propranolol on the ear skin temperature of cold exposed sheep

Experiment 3-8 was designed to investigate whether the β-antagonist propranolol would prevent peripheral vasodilatation in the ears of cold exposed sheep.

1. Experimental Animals

Four trials were performed (two per sheep) in which propranolol was administered either intravenously or intra-arterially, into one Suffolk x Cherviot wether, weight 30 kg and one Cheviot wether weight 60 kg. The sheep were housed and fed as described in chapter 2.

In the first two trials, injections were made via a jugular vein catheter as described in earlier experiments and in the latter two trials, injections were made via a carotid artery catheter introduced into an exteriorized carotid artery loop which had been surgically prepared by the method of Hecker (1974).

For each trial the animal was placed in a metabolic cage in a temperature controlled room in the company of another sheep in an adjacent cage. Heart rate electrodes, ear surface temperature thermocouples and a rectal probe were installed as described in chapter 2.

2. Experimental Protocol

At the end of a 30 min waiting period, the animals heart rate had stabilized, and the cooler which had been previously set to produce its' maximum cooling potential was turned on. The animals' environment was cooled until a "hunting response" was established as indicated by fluctuations in the dorsal surface temperature of the ear. The room temperature was -22.4 ± 1.2C at which time a dose of 0.5 mg/kg of a solution of propranolol (2 mg/ml) was injected either into the jugular vein or the carotid artery.

Ear surface temperature was measured throughout each experiment by a Honeywell model 10 millivolt recorder while heart rate, rectal temperature and room temperature were recorded, as previously described, before and after each injection.

3. Statistical Analysis

The results of experiment 3 - 8 were analyzed statistically by analysis of variance (Snedecor and Cochran 1967) and means were compared using Student Newman-Keuls' test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

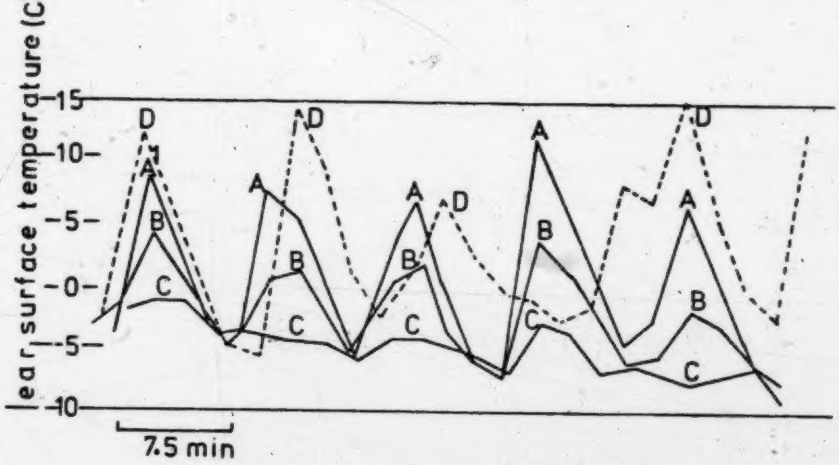
Experiment 3-1

The results of experiment 3-1 are illustrated in Figs. 7 and 8. Fig. 7 shows the recording of surface temperature from only three of the six thermocouples placed on the right ear (A. B and C) and one of the two thermocouples placed on the left ear (D) for an individual cold exposed sheep. These results were typical of all trials.

In every trial, all six sites on an individual ear "hunted" in synchrony. The most proximal site (C in Fig. 7) at the base of the ear maintained a low, relatively constant temperature with a minimal "hunting response". When it did show a warming response it did so in synchrony with the other sites on that ear. The "hunting responses" of the opposite ear were out of phase about 65 percent of the time (Fig. 7). This observation is not in agreement with observations made in cattle by Whittow (1962) who observed the surface temperature of the ears of cold exposed calves to "usually increase and decrease at the same time". He did note occasional asynchronous responses, however, the results obtained in the present study are in agreement with observations on the extremities of humans and sheep. For example Lewis (1930) and Greenfield _et al._ (1951b) reported synchronous hunting in adjacent fingers of man while Webster and Blaxter (1966) reported asynchronous

Figure 7

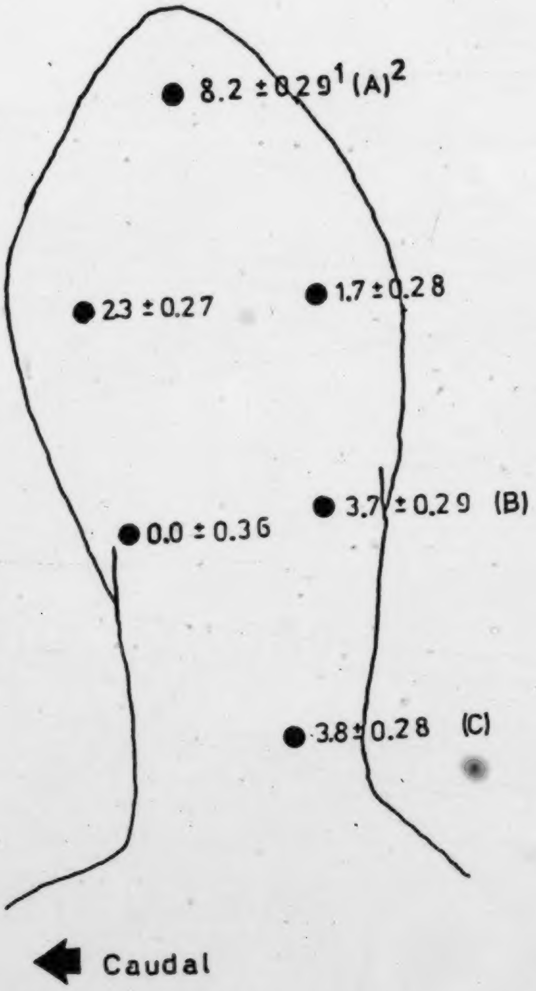
A recording of the ear skin temperature in a cold exposed (-18C) sheep.



[1] Three thermocouples (A,B and C, the solid lines) are on the right ear. One thermocouple (D the discontinuous line) is on the left ear.

Figure 8

The mean maximum temperature of the six thermocouples on the right ear of cold exposed sheep (X̄ ± SE).


8.2 ± 0.29[1] (A)[2]
2.3 ± 0.27
1.7 ± 0.28
0.0 ± 0.36
3.7 ± 0.29 (B)
3.8 ± 0.28 (C)
Caudal

1 all temperatures in degrees centigrade

2 letters represent the location of the thermocouples on the ear of the individual sheep reported in figure 7

hunting in the shanks and ears of sheep. The synchronous warming and cooling of the entire ear of the sheep observed in the present study is in agreement with the results of infrared photographic studies on jackrabbit ears (Hill and Veghte 1976). These authors suggested that vasoconstriction occurred in the major arteries of the ear, thus reducing the flow to the whole extremity.

During the constriction phase, the temperature of all sites on the ear surface of the sheep were similar. However, during each dilatation phase, the most distal thermocouple on the ear (A in Fig. 7) consistently showed the greatest degree of warming in all trials (Fig. 8). The direction of the thermal gradient observed during the "hunting response" was surprising since a counter current heat exchange between the warmer arterial blood and the returning cooler venous blood of the type described in the human arm by Bullard (1971) would be expected to create a gradient in the opposite direction. This observation confirms the studies of Aschoff (1944a and b) who found that the temperature increase was greatest in the terminal phalax of the fingers of a hand exposed to cold. In addition the observations illustrated in his papers of the first and second phalanax increasing in temperature simultaneously are consistent with the observations made in sheep ears in this experiment.

The vascular arrangement in the ears of sheep must be such that warm arterial blood flows rapidly to the tip of the pinna and venous blood returns through veins situated along both margins, to the base of the ear. As blood returns toward the base of the ear, it loses heat to the surface and the surrounding air.

The observation that the pinna warmed synchronously suggests that the control of the "hunting response" is perhaps located in the larger vessels of this extremity and is not located at the microcirculatory level. Thus, it would appear that a neural mechanism may be involved in the "hunting response". If a local control by metabolic end products as discussed by Haddy and Scott (1968) or a locally produced dilator as proposed by Hilton (1962) were the cause of this form of vasodilatation separate small areas within an extremity might be expected to warm independently as the concentration of these factors changed in the tissue surrounding the smaller vessels. If a circulating hormone was involved, all extremities would be expected to show synchronous "hunting responses" as the hormone concentration rose and fell within the general circulation. Since Meyer and Webster (1971) have shown that sympathetic innervation is required for vasoconstriction of the ear in the cold, the "hunting response" could possibly be caused by fluctuations in the activity of sympathetic nerve fibers to the blood vessels of a given extremity. This might occur in a manner similar to that shown by Iriki and Hales (1976) who observed an inverse relationship between the electrical activity of a postganglionic nerve twig and blood flow in one of the branchews of the retroauricular artery in the rabbit ear at a room temperature of 25C. If the "hunting response" is due to fluctuations in sympathetic neural activity, one might expect to observe significant fluctuations in concentrations of noradrenaline in plasma draining an extremity. Experiments 3-2, 3-3 and 3-4 were designed to measure the concentration of noradrenaline in plasma draining peripheral tissues in cold exposed sheep.

Experiment 3 - 2

Heart rate, rectal temperature and concentration of adrenaline and noradrenaline in arterial and venous blood from cold exposed sheep (-20.3C) are shown in Fig. 9 and Table 7. There were significant increases ($P<0.01$) in plasma adrenaline and noradrenaline concentrations and heart rate during the acute cold exposure. The results for venous plasma noradrenaline concentrations and heart rate agree in general with those reported by Thompson et al. (1978), however, adrenaline concentrations were slightly lower. The increased plasma noradrenaline concentrations probably reflect increased sympathetic neural activity. The

Figure 9

The plasma concentration of noradrenaline and adrenaline in blood samples taken simultaneously from the right carotid artery and the right jugular vein in cold exposed (-20.3C) sheep

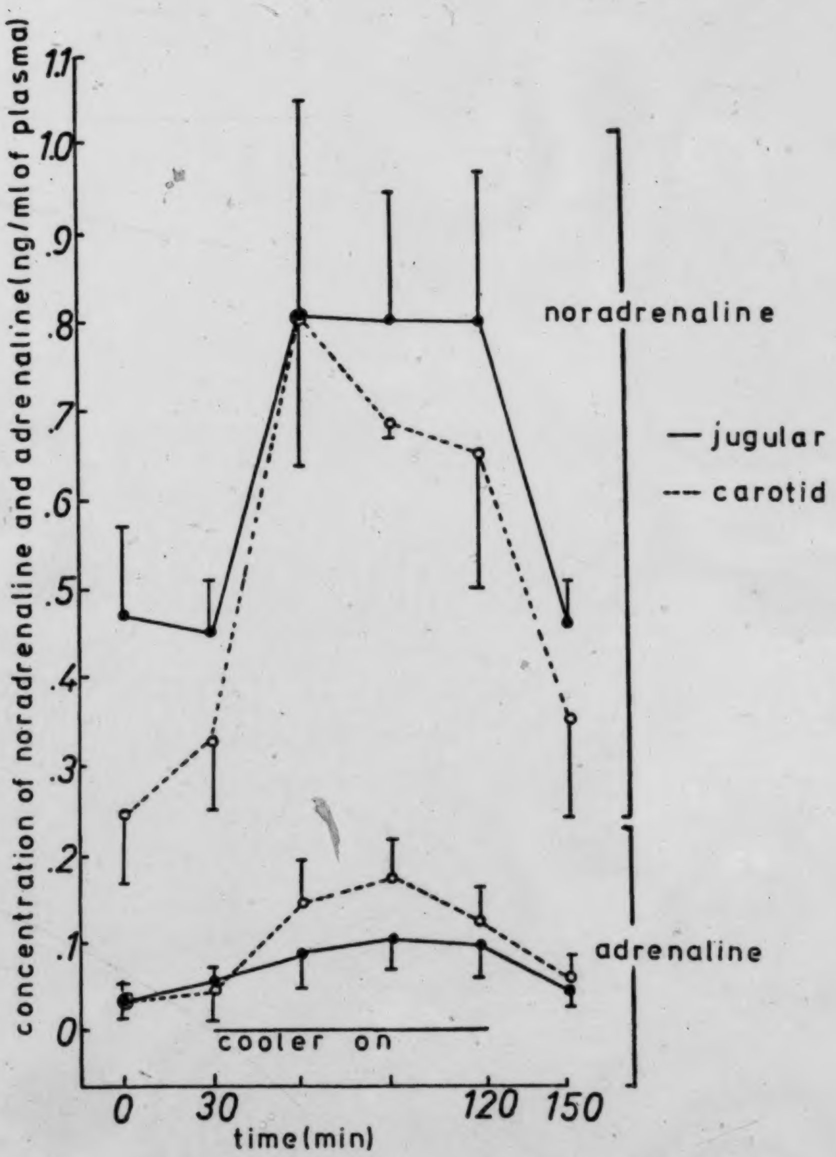


Table 7. The effect of acute cold exposure on arterial and venous plasma concentrations of adrenaline and noradrenaline, heart rate, and rectal temperature in sheep. Experiment 3-2.

		Adrenaline Concentration		Noradrenaline Concentration			
Time (min)	Room temp. (C)	Arterial (ng/ml)	Venous (ng/ml)	Arterial (ng/ml)	Venous (ng/ml)	Temperature (C)	Heart rate (beat/min)
0	23.7	0.04^{c,1}	0.04^{c}	0.24	0.47	39.9	93.5^{a}
30	24.6	0.05^{c}	0.05^{c}	0.33	0.45	39.9	94.0^{a}
(Cooler on)							
90	-20.3	0.15^{ab}	0.09^{bc}	0.80	0.81	39.9	164.5^{b}
120	-20.9	0.17^{a}	0.10^{abc}	0.68	0.80	39.5	199.0^{b}
150	-21.5	0.12^{abc}	0.10^{abc}	0.66	0.80	39.6	194.5^{b}
(Cooler off)							
240	14.1	0.05^{c}	0.04^{c}	0.35	0.45	40.0	103.0^{a}
SE^{2}		0.016		0.14		0.10	14.6

[1] The number of observations per treatment is four.

[2] SE-Standard error of the means.

[a,b,c] Means with different superscripts are significantly different ($P<0.05$) for adrenaline concentrations and for heart rate.

increased plasma adrenaline concentrations probably reflect increased adrenal medullary activity as shown in dogs by Klepping _et al._ (1957). Similarly, Leduc (1961a and b) showed an immediate six fold increase for adrenaline excretion in rats when exposed to an environmental temperature of 3C.

In the present study the increase in plasma adrenaline concentration was significantly greater in arterial than in venous blood. A significant interaction ($P<0.05$) between arterio-venous difference and time period was observed. This indicates that arterial adrenaline concentration increased in the cold to a greater extent than the venous concentration possibly reflecting an increased uptake and utilization of circulating adrenaline by the tissues of the head. Since Grodsky (1973) states adrenaline does not readily cross the blood brain barrier it is therefore likely these results reflect uptake by tissues outside of the brain.

The arterio-venous differences in noradrenaline concentration were not significant ($P<0.05$). When arterial versus venous noradrenaline concentrations were compared for the samples taken during the cold period alone, again no statistically significant difference ($P<0.05$) existed. This would indicate that the net contribution of the head to circulating noradrenaline was small under the conditions of this experiment. The lack of a significant arterio-venous difference in noradrenaline concentration during cold exposure was somewhat surprising. The major mechanism of noradrenaline removal from sympathetically innervated tissue is uptake by the sympathetic nerve endings (Iverson 1967) while some is also degraded enzymatically in the tissues as discussed by Shepherd and Vanhoutte (1975) and a small portion representing an overflow diffuses into the blood. Since there was an increased plasma concentration of noradrenaline during cold it could be possible, with sufficient replication, to detect a significant arterio-venous difference in concentration across tissues with high sympathetic neural activity. However the ears were generally dilating during at least a portion of each blood sampling period. If dilatation is due to a reduced output of noradrenaline from the adrenergic nerves, there would likely be a reduction in the arterio-venous difference during dilatation. Therefore, sampling from the jugular vein during periods of fluctuating ear surface temperature would be expected to increase the variability in arterio-venous concentration differences.

Experiment 3 - 3

The results of the two trials in which blood samples were collected simultaneously from the jugular veins on the intact and sympathectomized sides of the head of a cold exposed sheep are presented in Figs. 10, 11, 12 and Table 8. Cold exposure significantly increased ($P<0.05$) plasma noradrenaline and adrenaline concentrations. The difference between arterial and venous plasma noradrenaline concentrations was not significant ($P<0.05$). As the sympathectomy would be expected to reduce the outflow of noradrenaline from that side of the head, the lack of a significant difference in these data may be due to the small number of observations. On the other hand, as the blood vessels on the sympathectomized side of the head remained dilated throughout the cold exposure (Fig. 12) the resistance to blood flow would be lower on this side. This might increase the percentage of blood returning via collateral circulation from the opposite side of the head into the jugular vein of the sympathectomized side. This factor would tend to reduce any difference in noradrenaline concentration between the jugular veins on the intact and sympathectomized sides.

The mean plasma adrenaline concentration was not statistically significantly different ($P<0.05$) between the intact and sympathectomized sides of the head. If there was

Figure 10

The plasma concentration of adrenaline in blood samples taken simultaneously from the jugular veins on the sympathectomized and intact sides of the head in cold exposed (-17.5C) sheep

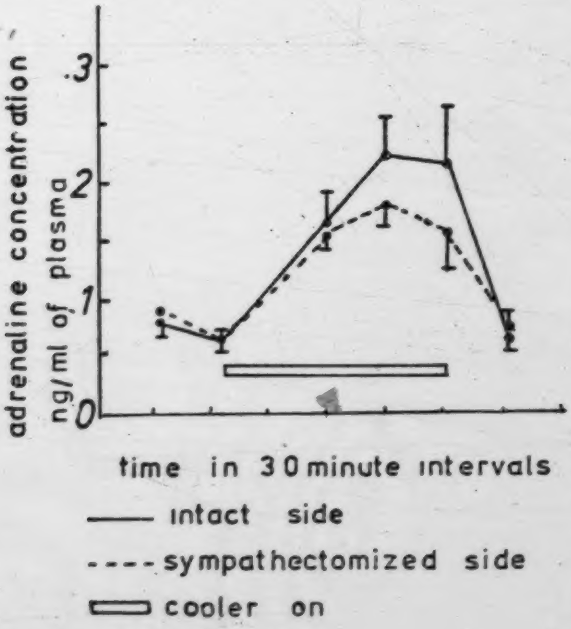


Figure 11

The plasma concentration of noradrenaline in blood samples taken simultaneously from the jugular veins on the sympathectomized and intact sides of the head in cold exposed (-17·5C) sheep

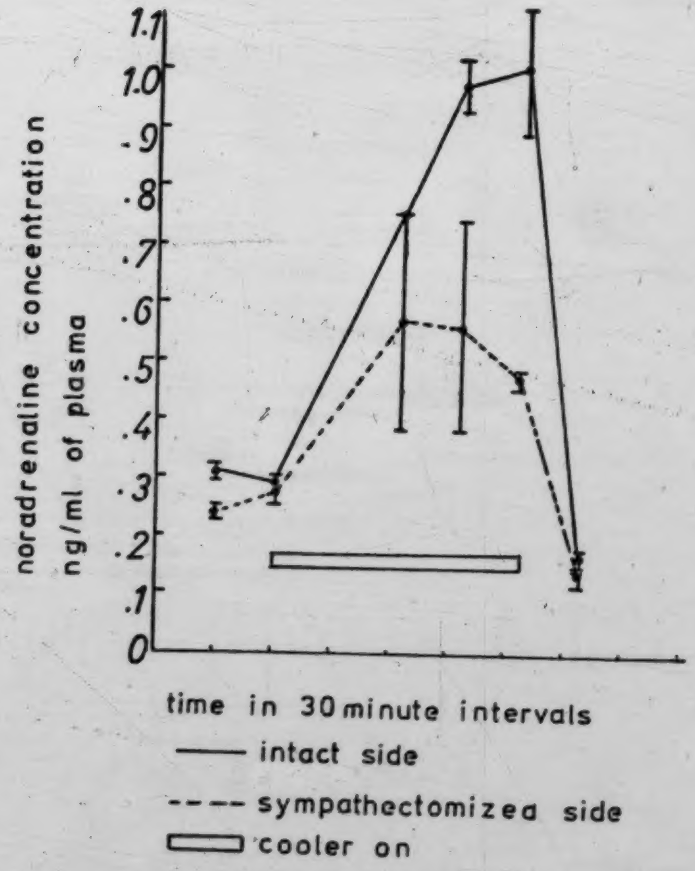


Figure 12
An example of the skin surface temperature from the dorsal surface of the ears on the intact and sympathectomized sides of the head in a cold exposed (-20C) sheep

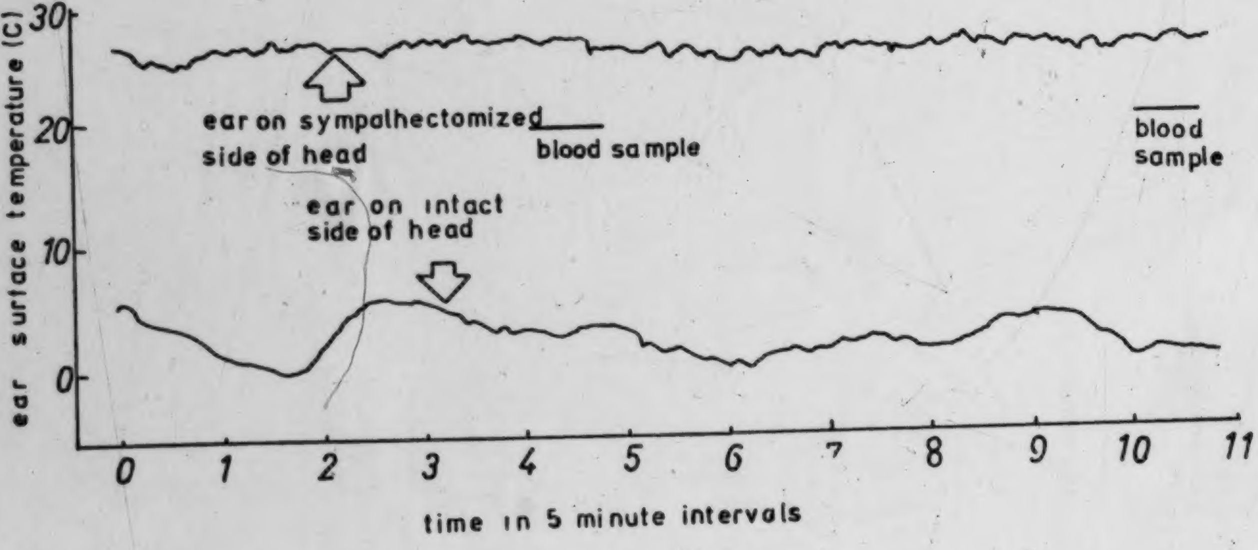


Table 8. The effect of sympathectomy on venous plasma concentrations of adrenaline and noradrenaline, hematocrit and heart rate in cold exposed sheep. Experiment 3-3.

Room temp. (C)	Adrenaline (ng/ml)		Noradrenaline (ng/ml)		Hematocrit %		Heart rate (beat/min)
	Intact	Sympath.	Intact	Sympath.	Intact	Sympath.	
21.2	0.07[1]	0.08	0.32	0.24	26.1^{a}	27.6^{a}	112^{a}
24.3	0.07	0.07	0.28	0.28	22.3^{a}	25.2^{a}	94^{a}
-15.8	0.12	0.15	0.76	0.58	29.3^{b}	30.7^{b}	196^{b}
-17.5	0.22	0.17	0.97	0.56	28.5^{b}	29.5^{b}	204^{b}
-17.0	0.21	0.15	1.00	0.47	28.2^{ab}	29.0^{b}	192^{b}
16.8	0.06	0.07	0.16	0.13	23.0^{a}	23.3^{a}	108^{a}
SE[2]	0.05		0.25		1.2		9.2

[1] The number of observations per mean is two.

[2] SE-Standard error of the means.

[a,b] Means within colums with different superscripts are significantly different ($P<0.05$).

increased capillary flow in the dilated sympathectomized side of the head the slightly lower plasma concentration of adrenaline may reflect increased tissue uptake and/or enzymatic breakdown on that side of the head.

Experiment 3 - 4

Jugular plasma concentrations of noradrenaline and adrenaline during the dilating and constricting phases of individual "hunting responses" are shown in Table 9. A statistically significant difference ($P<0.05$ for noradrenaline and $P<0.01$ for adrenaline) between the dilating and constricting phases was found for both plasma adrenaline and noradrenaline concentrations. The noradrenaline concentration was significantly higher ($P<0.05$) in the constriction phase than in the dilatation phase of the "hunt". This could indicate that during the dilatation phase, decreased sympathetic neural activity resulted in a decreased outflow of noradrenaline into the circulatory system. This would support the suggestion that the "hunting response" is due to relaxation of sympathetic tone. However, the results could also indicate that the increased blood flow during the "hunting response" diluted a constant outflow of noradrenaline. Thus while the data are consistent with the idea of relaxation of sympathetic tone, they do not provide conclusive evidence that this mechanism causes the "hunting response".

There was also a consistent increase in plasma adrenaline concentration during the constriction phase of the "hunting response" ($P<0.01$). This might indicate that there was an increase in adrenal medullary activity during

Table 9. Concentrations of adrenaline and noradrenaline in plasma from the right jugular vein during the dilatation and constriction phases of individual "hunting responses" in the right ear of cold exposed sheep. Experiment 3-4.

Animal No.	Room Temperature (C)	"Hunting Response"	T_{SD}[1] (C)	T_{SC}[2] (C)	Adrenaline (ng/ml) Dilatation Phase	Adrenaline (ng/ml) Constriction Phase	Percent Change	Noradrenaline (ng/ml) Dilatation Phase	Noradrenaline (ng/ml) Constriction Phase	Percent Change
577	-15.0	1	5.6	7.6	0.30	0.54	+73.3	1.06	0.10	+ 3.8
		2	10.8	3.2	0.33	0.48	+45.5	1.15	1.33	+15.7
582	-20.0	1	4.3	8.7	0.02	0.04	+100.0	0.35	0.51	+45.7
		2	5.9	11.6	0.02	0.03	+50.0	0.34	0.39	+14.7
		3	8.1	6.0	0.04	0.07	+75.0	0.32	0.43	+34.4
		4	12.4	7.6	0.02	0.03	+50.0	0.37	0.43	+16.2
		5	5.4	11.8	0.04	0.05	+25.0	0.32	0.39	+21.9
		Mean	7.5	8.1	0.11	0.17*	+49.4	0.56	0.65*	+21.7
		SE[3]			0.01			0.007		

[1] T_{SD} = increase in skin temperature (C) while sampling during the dilatation phase.

[2] T_{SC} = decrease in skin temperature (C) while sampling during the concentration phase.

[3] SE-standard error of the mean.

* Denotes a significant difference between the mean values for the constriction and dilatation phase ($P<0.05$ for noradrenaline, $P<0.01$ for adrenaline).

the constriction phase. On the other hand, it may reflect an increased tissue uptake and/or enzymatic breakdown during the dilatation phase. If there was increased capillary flow during dilatation as previously stated there would be greater opportunity for tissue uptake of adrenaline. Although adrenaline can act on β receptors of vascular smooth muscle as shown by Sicuteri et al. (1966) , Del Bianco et al. (1972), and Shepherd and Vanhoutte (1975), and has been shown to induce vasodilatation in the tail of the rat by Fregly et al. (1976) it is unlikely that circulating adrenaline is the cause of the dilatation phase of the "hunting response". A circulating hormone would be expected to cause synchronous "hunting" in all extremities. The results show that there was a greater concentration of adrenaline in the jugular plasma during the constriction, rather than during the dilatation phase. This would not be consistent with a dilatory role for adrenaline. Adrenaline might, however, have a role in enhancing release of noradrenaline from sympathetic nerve endings. Westfall (1977) suggests that adrenaline has been shown to enchance the release of noradrenaline from nerve endings during nerve stimulation. Sjtarne and Brundin (1976) have shown that increased nerve stimulation induced secretion of ^3H noradrenaline in the presence of isoprenaline (isoproterenol) a β agonist. This increased secretion could be blocked by the specific β_2 antagonist H 35/25 while two specific β_1 blocking agents could not prevent this response. This lead Westfall (1977) to hypothesize that the β_2 prejunctional receptors react to circulating levels of adrenaline to increase the output of noradrenaline from sympathetic nerve endings. Burton and Edholm (1955) and Greenfield (1963) noted that, in humans, cold induced vasodilatation is often preceded by a sensation of pain. Perhaps the increased plasma adrenaline concentrations during the constricted phase reflect discomfort associated with the "hunting response" in the ears of the sheep.

Experiment 3 - 5

Seven acute (120 min) trials were performed in which the cervical sympathetic nerve was severed and the cranial portion stimulated in anaesthetized sheep. Carotid artery and posterior auricular venous blood samples were collected during the last three trials and plasma noradrenaline concentrations were determined (Table 10). In two of the seven trials, "hunting responses" were observed in the cooled ear prior to severing the cervical sympathetic nerve. An example for an individual trial is shown in Fig. 13. The difficulty in eliciting a "hunting response" in anaesethetized animals has been previously noted by Edwards (1967) and Schwinghamer and Adams (1969).

Severing the nerve trunk always resulted in an increase in skin temperature reflecting a vascular dilatation. Stimulation of the cranial portion of the nerve caused a decrease in skin temperature, presumably due to vasoconstriction. However, difficulty was encountered in several trials in maintaining a constant, uniform contact between the nerve and the stimulating electrodes. This resulted in periodic warming of the ear surface as shown in Fig. 14 which, in several cases, gave a pattern resembling cold induced vasodilatation. In some cases, the pattern differed markedly from the cold induced vasodilatation. In every case, adjustment of the position of the electrodes or

Table 10. The effect of severing the cervical sympathetic nerve and electrical stimulation of the cranial portion of the nerve on carotid artery and auricular vein noradrenaline concentration during cooling of an ear. Experiment 3-5, trials 5, 6 and 7.

Ear Bath Temp.	NA concentration (ng/ml) Carotid artery	Auricular Vein	A-V Diff.	Condition of ear	Nerve[1]	Direction of ear temp[2]	Stimulator[3]
Trial 5							
warm	0.033	0.106	- .073	dilated	+	→	-
cold	0.582	0.483	+0.099	partially constricted	+	→	-
cold	0.610	0.592	+0.018	partially constricted	+	↘	-
cold	0.532	0.440	+0.092		+	↗	-
cold	0.311	0.716	-0.385	dilated	-	↘	-
cold	0.669	1.156	-0.487	constricted	-	↘	+
cold	0.879	1.549	-0.670	constricted	-	↘	+
Trial 6							
warm	0.130	0.215	-0.085	dilated	+	→	-
cold	0.173	0.120	+0.053	constricted	+	→	-
cold	0.409	0.806	-0.397	constricted	+	↘	-
cold	0.356	0.273	+0.083	dilated	-	→	-
cold	0.408	0.821	-0.413	constricted	-	↘	+
cold	0.487	0.999	-0.512	constricted	-	↘	+
cold	0.334	0.424	-0.080	constricted	-	↗	+
cold	0.232	0.713	-0.481	constricted	-	↘	+
Trial 7							
warm	0.158	0.251	-0.093	dilated	+	→	-
warm	0.206	0.247	-0.041	dilated	+	→	-
cold	0.368	0.453	-0.085	partially constricted	+	↗	-
cold	0.671	0.813	-0.141	partially constricted	+	→	-
cold	0.375	0.357	+0.016	dilated	-	→	-
cold	0.627	1.187	-0.515	constricted	-	↘	+
cold	0.608	1.025	-0.416	constricted	-	↘	+
cold	0.496	0.815	-0.319	constricted	-	↘	+

[1] Nerve + = nerve intact, - = nerve cut.

[2] Direction of ear temperature → = ear temperature steady, ↗ = ear temperature increasing, ↘ = ear temperature declining.

[3] Stimulator + = electrical stimulator on, - = electrical stimulator off.

Figure 13

An example of the ear skin temperature from the dorsal surface of a cooled (0 C) ear to which the cervical sympathetic nerve was cut and the cranial portion electrically stimulated

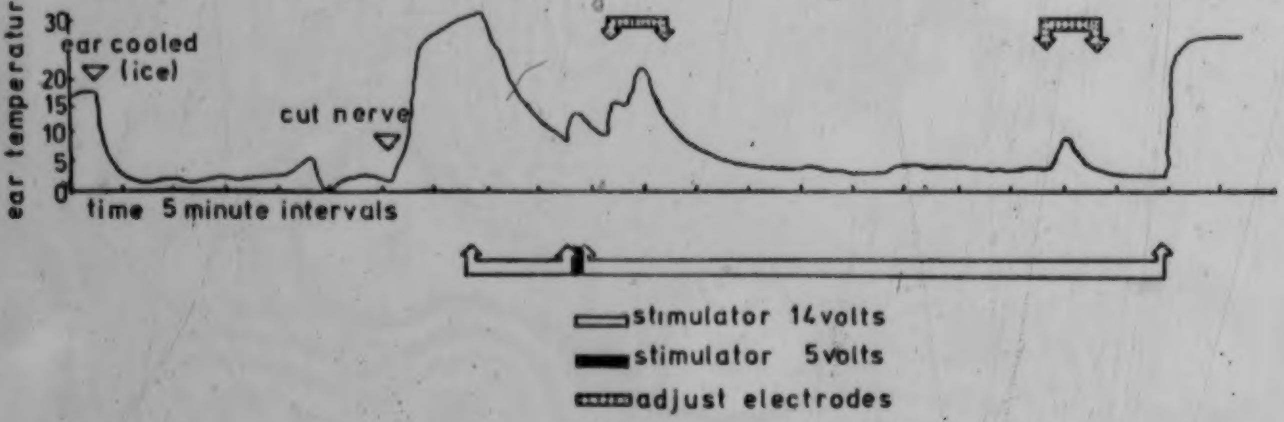
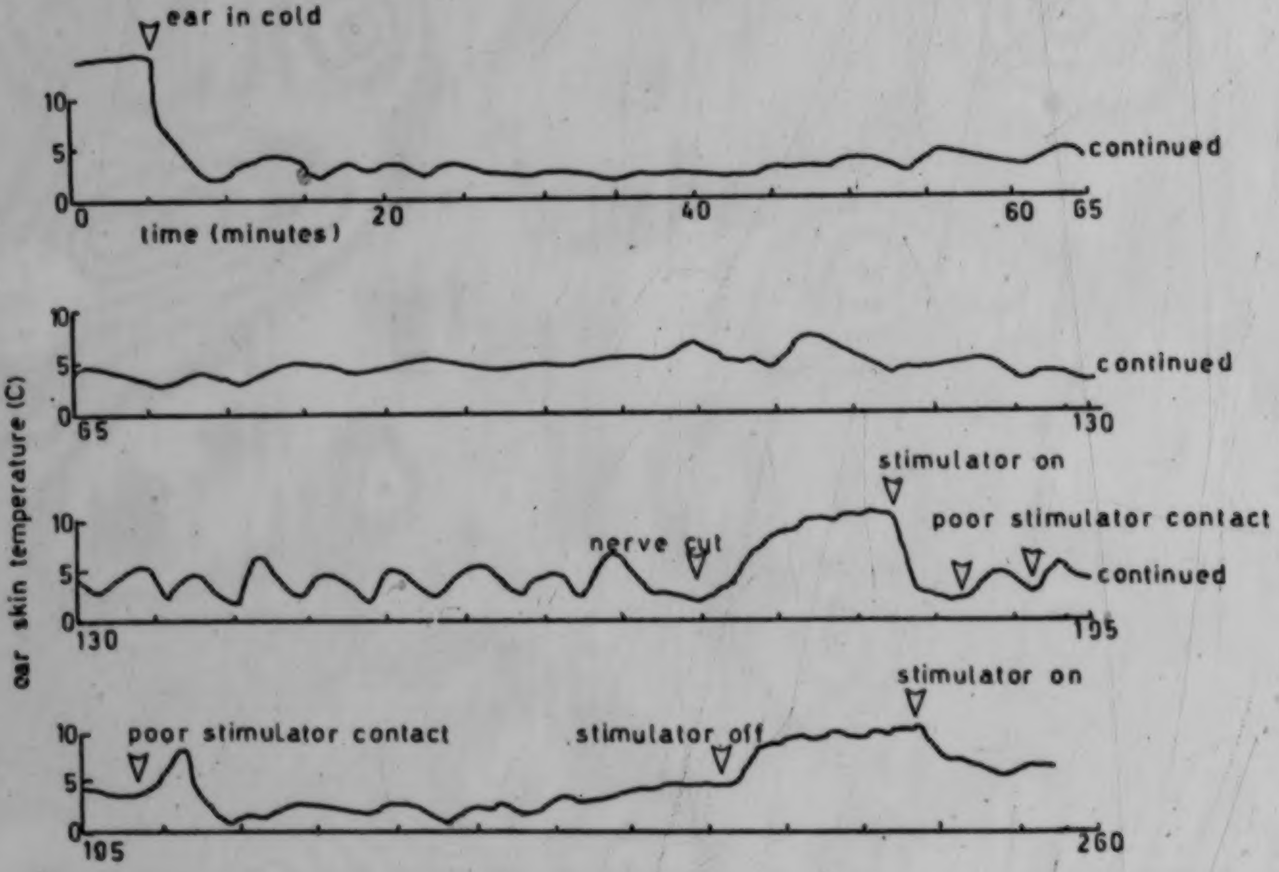


Figure 14
An example of the ear skin temperature from the dorsal surface of a cooled ear(-2C) to which the cervical sympathetic nerve was cut and the cranial portion electrically stimulated

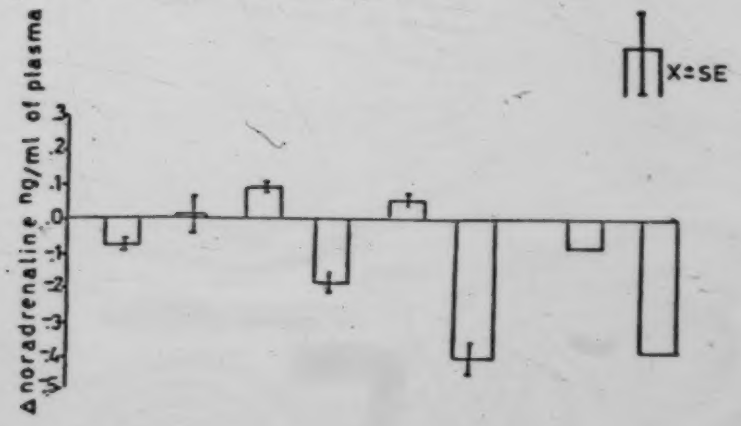


an increase in stimulation voltage resulted in a sustained decrease in ear skin temperature. As long as the stimulator voltage was high enough (14 to 15 V) and as long as good contact was maintained there was no "hunting response" during nerve stimulation. This suggested that the "hunting response" may be due to periodic interruption of sympathetic neural activity to an extremity, rather than to the build up of a dilator.

The arterio-auricular venous differences in plasma noradrenaline concentrations shown in Table 10 were summarized into eight categories (cases) as described in Fig. 15. When the ear was dilated and not showing signs of constricting, either in the warm, before cold immersion (Case 1, Fig. 15), or in the cold bath after nerve severance (Case 5, Fig. 15) there was only a slight arterio-auricular venous difference in plasma noradrenaline concentration. Likewise, when the constricted ear was either maintaining a constant temperature (Case 2, Fig. 15) or dilating (Case 3, Fig. 15) there was little or no arterio-auricular venous difference in plasma noradrenaline concentration. When the constricted ear with an intact sympathetic nerve supply was further constricting (Case 4, Fig. 15) there was a large negative arterio-auricular venous difference in plasma noradrenaline concentration. In cases where the constricted ear was further constricting

Figure 15

The effect of cutting the cervical sympathetic nerve to a cooled (OC) ear and electrically stimulating the cranial portion on the carotid artery minus the auricular vein plasma noradrenaline in anesthetised sheep



number of observ^{1}	4	3	2	2	2	8	1	1
dilated or constr.^{2}	D	C	C	C	D	C	C	D
environment^{3}	w	c	c	c	c	c	c	c
nerve^{4}	+	+	+	+	−	−	−	−
direction^{5}	>	>	7	↘	>	↘	7	↘
stimulation^{6}	−	−	−	−	−	+	+	+
case	1	2	3	4	5	6	7	8

1. n = number of observations
2. D = dilated , C = constricted
3. w = warm , c = cold
4. + = nerve intact, − = nerve severed
5. > = ear stable , 7 = ear warming , ↘ = ear cooling
6. − = electrical stimulation off , + = electrical stimulation on

under the influence of electrical stimulation of the severed sympathetic nerve (Case 6, Fig. 15), there was an even larger negative arterio-auricular venous difference in plasma noradrenaline concentrations. On one occasion, the ear showed signs of dilatation while the stimulator was on (Case 7, Fig. 15). This was associated with a small arterio-auricular venous difference in plasma noradrenaline concentration, however, and could be attributed to poor contact between the stimulating electrodes and the nerve. On another occasion, when the sympathetic nerve was not being stimulated, the dilated ear showed a large negative arterio-auricular venous difference in plasma noradrenaline concentration (Case 8, Fig. 15). This was accompanied by a brief decrease in ear temperature, suggesting that the recently severed nerve may have fired spontaneously. It is also possible that the nerve may have fired in response to a mechanical stimulation caused by a shift in position of the portion of syringe barrel holding the electrodes and the nerve.

The results of these trials suggest that the "hunting response" may be due to fluctuations in the release of noradrenaline from the sympathetic nerves supplying the blood vessels of an extremity, since there was a large arterio-venous difference in noradrenaline concentration across the ear only at times when the ear temperature was declining. The "continuous proportional control" type of response mentioned above might also be due to fluctuations in noradrenaline release from sympathetic nerve endings. This suggestion is supported by the observation that arterio-venous differences in noradrenaline were small at times when the ear was constricted but maintaining a steady temperature (Case 2, Fig. 15).

While fluctuations in sympathetic neural activity may be involved in cold induced vasodilatation, there remains the question as to what mechanism might selectively control the sympathetic neural activity of individual extremities. Westfall (1977) has reviewed mechanisms that might regulate adrenergic transmission by altering the noradrenaline output from the nerve ending. Several mechanisms that might result in temporary reduction in adrenergic transmission are as follows: (a) inhibition of noradrenaline release by high concentrations of noradrenaline itself, (b) inhibition of noradrenaline release by substances produced locally by the target tissue such as PG as shown in the rabbit ear artery by Hadazy (1976), (c) contralateral neuronal control in which release of acetylcholine from nerves that terminate near adrenergic endings inhibits noradrenaline release, and (d) control by locally formed or blood borne mediators such as histimine or dopamine.

In addition to inhibitory factors, there are several substances which are possible enhancers of noradrenaline release. Two compounds have been suggested to facilitate the release of noradrenaline from sympathetic nerve endings according to the review by Westfall (1977). The first compound, adrenaline, which may activate β adrenergic receptors on adrenergic nerve endings has already been discussed. The second compound suggested to effect the release of noradrenaline was angiotensin. Angiotensin was considered to act by enhancing release of noradrenaline rather than by inhibiting re-uptake of noradrenaline as it has been shown by several authors (Zimmerman and Gomez 1965, Bell and McLenn 1967, Zimmerman and Gisslen 1968 and Kadowitz et al. 1971c) that angiotensin increases the response to exogenous noradrenaline.

Although no definitive evidence exists that physiological levels of angiotensin modify vasomotor tone, Westfall (1977) suggested that angiotensin will facilitate adrenergic neurotransmission. Renin has been shown to be present in the blood vessels of rats (Rosenthal et al. 1969). A synthetic tetradecapeptide renin substrate and purified hog renin potentiated vasoconstrictor response to sympathetic stimulation of the isolated rat mesenteric artery (Malik and Najletti 1976). This response was prevented by an inhibitor of the angiotensin I converting enzyme SQ20881 and an angiotensin II receptor antagonist

(Sar'Ile) AII. This suggested to Westfall (1977) the utilization of renin substrate within the vascular wall by renin or reninlike enzymes resulted in the formation of angiotensin I which can be converted to angiotensin II which, in turn, enhances the vasopressor response by augmenting norepinephine release. These ideas are consistent with the idea that the local generation of angiotensin II in blood vessels may play a role in modulating sympathetic nerve activity. Clearly, there are a multitude of factors which could conceivably be involved in modulation of the release of noradrenaline from sympathetic nerves. The situation is further complicated by the fact that most of the substances mentioned above which influence noradrenaline release also have direct effects on smooth muscle.

Experiment 3 - 6

The injection of isoproterenol (0.2 µg/kg) was sufficient to significantly increase ($P<0.05$) heart rate (Table 11) from 156 ± 23 beats/min to 231 ± 18 beats/min. Injection of propranolol (0.5 mg/kg) produced no significant ($P<0.05$), depression in heart rate. Following the propranolol treatment, injection of isoproterenol did not induce a change in heart rate at 0 to 20 min post injection (Table 11) suggesting the β blocking action of propranolol on the heart was complete.

There was no significant effect of isoproterenol on the maximum and minimum skin temperatures or on the maximum minus minimum temperature or the interval between "hunting responses" before or after the propranolol (Table 11). These results suggest that activation of β receptors on vascular smooth muscle is not a probable cause of cold induced vasodilatation. On the other hand, there is the possibility that in this experiment, an insufficient amount of isoproterenol reached the constricted ears to cause dilatation. The systemic dose of 0.2 µg/kg isoproterenol appeared to induce maximal heart rate responses as seen in Table 11. To avoid the possibility of inducing serious cardiac arythmias in subsequent experiments higher dose rates were administered subcutaneously intc the ear tissue rather than intravenously.

Table 11. The mean maximum, minimum, maximum minus minimum ear skin temperature, interval between "hunting responses", heart rate and rectal temperature before and after intravenous isoproterenol and propranolol in cold exposed (-17±1.5C) sheep. Experiment 3-6.

	Isoproterenol Dose (μg/kg)	Maximum (C)	Minimum (C)	Difference (C)	Interval (s)	Heart Rate (beat/min)	Rectal Temperature (C)
Before propranolol	0	5.6(14)[1]	0.5(14)	5.1	417 (11)	156.7[a]	38.7
	0.2	7.5(11)	1.3(11)	6.2	452 (8)	231.3[b]	38.7
After propranolol (0-20 min)	0	6.2(13)	1.4(13)	4.9	588 (10)	102.3[a]	39.1
	0.2	6.5(7)	2.4(7)	4.8	429 (4)	100.6[a]	39.1
(60-90 min)	0.2	6.7(14)	0.9(14)	5.9	521 (13)	131.6[a]	39.0
SE[2]		0.76	1.7	1.7	32	12.6	0.13

[1] Number of observations per mean.

[2] SE-Standard error of the mean.

[a,b] -Means with different superscripts differ significantly ($P<0.05$).

Experiment 3 - 7

Table 12 and Fig. 16 show the effects of subcutaneous injections of increasing amounts of the β agonist isoproterenol on the dorsal skin surface temperature of the ears in cold exposed (3C) sheep. The isoproterenol dose levels of 100 to 400 μg were sufficient to cause statistically significant ($P<0.05$) increases in the heart rate of the sheep as shown in Fig. 17. However, no change was found in the surface temperature of the cold exposed ears. The concentration of isoproterenol in blood, although sufficient to stimulate the heart, would likely be several fold lower than the concentration within the ear tissue following subcutaneous injection. These results suggest that relatively little or no β adrenergic dilator activity occurs in the blood vessels of the ears in sheep.

Table 12. The mean ear skin temperature and heart rate following increasing subcutaneous doses of isoproterenol in cold exposed (3-2C) sheep. Experiment 3-7.

	Dose (µg)	Before Isoproterenol	After Isoproterenol	SE[2]
Ear skin temperature (C)	100	5.4[1]	4.9	
	200	4.0	3.4	
	400	6.7	6.6	0.37
Heart rate (beat/min)	100	65.0[a]	71.5[b]	
	200	64.0[a]	73.0[b]	
	400	73.3[a]	89.0[b]	5.3

[1] The number of observations per mean is three.

[2] SE - Standard error of the means.

[a,b] Means with different superscripts within a row differ significantly (P<0.05).

Figure 16
The effect of subcutaneous injection of increasing doses of isoproterenol on ear surface temperature in cold exposed (-3C) sheep

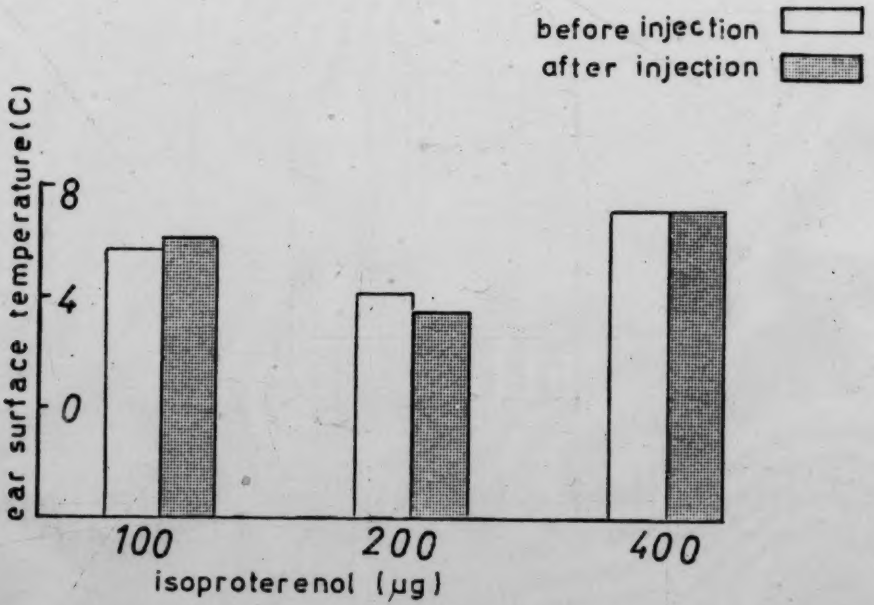
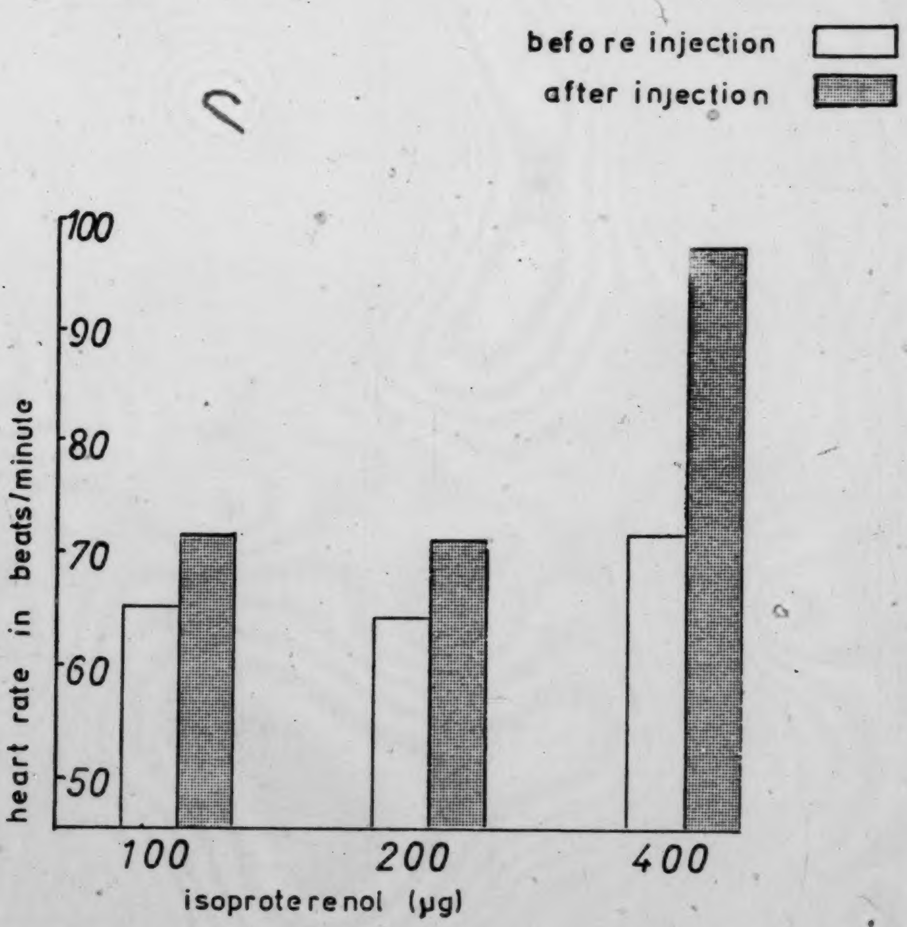


Figure 17

The effect of subcutaneous injection of increasing doses of isoproterenol on heart rate in cold exposed (-3C) sheep



Experiment 3 - 8

The results of the four trials in which propranolol (0.5 mg/kg) was infused either by the jugular vein, or the carotid artery are shown in Table 13. No significant differences due to treatment were found in the maximum ear skin temperature, minimum ear skin temperature, the maximum minus minimum ear skin temperature or the interval between "hunting responses". This data together with the data from experiments 3 - 6 and 3 - 7, suggest that activation of β adrenergic receptors is probably not the cause of the "hunting response". The results of the present experiments are not consistent with those of Fregly *et al.* (1976) who reported dilatory effects of isoproterenol on the tail skin in rats. In the experiments of Fregly *et al.* (1976) the total dose rate per kg was much higher (50 - 100 µg/kg) than in the present study. However, in experiment 3 - 7, the direct subcutaneous injection of 400 µg of isoproterenol in sheep, would be expected to result in local ear tissue concentrations at least as high as those achieved in the tail skin of the rat. It, therefore, seems unlikely that a β adrenergic dilatory mechanism exists in the cutaneous blood vessels of sheep.

The infusion of 0.5 mg/kg of propranolol reduced the heart rate ($P<0.05$) in each of the four trials. This is in agreement with the findings of Webster and Hays (1968). There was no effect on rectal temperature.

Table 13. The mean maximum, minimum, maximum minus minimum ear skin temperature, interval between "hunting responses", heart rate and rectal temperature before and after jugular vein or carotid artery infusion of 0.5 mg/kg propronolol in cold exposed (-22.4±1.2C) Sheep. Experiment 3-8.

	Ear Skin Temperature (C)																	
Site of Infusion	Maximum		SE[1]	Minimum		SE	Maximum-Minimum		SE	Interval(s)		SE	Heart Rate (beat/min)			Rectal Temperature (C)		
	before (C)	after (C)		before (C)	after (C)		before (C)	after (C)		before (C)	after (C)		before	after	SE	before	after	SE
Jugular	7.4[2]	7.5	0.6	1.3	2.3	0.4	6.1	5.3	0.7	469	414	44	155[a]	105[b]	22.0	39.7[3]	39.8	0.07
Carotid	5.7	5.0	0.4	-1.3	-2.4	0.3	7.0	7.3	0.8	333	335	26	175[a]	115[b]	19.5	38.8	38.6	0.26

[1] SE-Standard error of the mean.

[2] The harmonic mean of the number of observations per treatment was 6.00 for the interval and 8.40 for the maximum, minimum and maximum minus minimum ear skin temperature.

[3] The number of observations per mean is two for both heart rate and rectal temperature.

[a,b] Means with different superscripts within rows are statistically significant ($P<0.05$).

CONCLUSIONS

The skin of cattle and sheep contains large numbers of mast cells which are found in groups surrounding blood vessels, sebaceous glands and within the connective tissue surrounding the hair follicles. The number of mast cells is greatest in the papillary layer of the skin and declines toward the deeper reticular layer. The higher density of the mast cells in the more superficial areas of the dermis would suggest a role for these cells in the response of the skin to changes in the external environment. Whether this role is restricted to participation in the inflammatory response to tissue injury (Selye 1965) or whether it involves participation in responses of the skin to more moderate environmental stimulation, such as air temperature change, remains to be elucidated. Though temperature did not appear to affect mast cell numbers in cattle and sheep in this study, the relatively large numbers present in the skin and the close anatomical association between mast cells and blood vessels suggests the involvement of mast cells in the control of blood flow in response to environmental temperature changes. Histamine, one of the compounds released from mast cell granules, has vasodilator properties in the skin. Experiments described in chapter 2 indicate that the cold constricted ears of sheep will warm in response to local subcutaneous injection of histamine and that low doses of an H_1 receptor blocking agent will reduce this response. However, similar doses of H_1 receptor blocking agent do not affect the naturally occurring "hunting response" in cold exposed sheep, suggesting that the "hunting response" in sheep is not mediated by an H_1 dilator mechanism.

The lack of reduction of the dilatory responses to exogenous histamine following high doses of the H_1 blocking agent, suggests that there may be two populations of H_1 receptors having different affinities for histamine. The results can be interpreted as evidence for a high affinity pre-junctional H_1 dilator receptor possibly on the sympathetic nerve terminal which is blocked by low doses of H_1 blocking agent and a low - affinity post junctional H_1 constrictor receptor possibly on the smooth muscle cells which is blocked by high doses of H_1 blocking agent. In addition, there clearly must be another histamine receptor which mediates a dilatory response in the presence of high doses of the H_1 blocking agents. This may be an H_2 type of histamine receptor. The single dose rate of H_2 blocking agent used in the present study did not influence skin temperature responses to histamine or the naturally occurring "hunting response". However, higher doses of the H_2 blocking agent might be required to satisfactorily block these receptor sites. Further studies are therefore needed to determine the possible role of H_2 receptors in the "hunting response".

The entire surface of an individual ear was shown to warm and cool synchronously with the most distal portion of the ear, reaching the highest temperature during a given "hunting response". Therefore, the control of the "hunting response" in the ears of sheep may be vested in the control of the larger vessels rather than the smaller vessels serving different localized areas within the ear. This lends support to the hypothesis that changes in neural activity to the major blood vessels of an extremity may be the cause of the "hunting response".

The β adrenergic agonist isoproterenol and the β adrenergic antagonist, propranolol, did not affect the "hunting response" in the ears of sheep. This suggests that these periodic dilatations are not caused by the activation of β adrenergic receptor sites.

Severing the sympathetic nerve to one side of the head always resulted in an increased ear skin temperature on the same side. Continuous electrical stimulation of the cranial portion of the nerve resulted in a constriction which was sustained as long as there was no interruption of nerve stimulation. Thus, continuous electrical stimulation of the nerve prevented the "hunting response" but did not result in a complete cessation of blood flow or freezing of the ear tissues. In a similar manner, the infusion of noradrenaline was seen to prevent hunting in an intact ear of sheep by Meyer and Webster (1971). Sheep in a cold environment are, therefore, capable of maintaining sufficient blood flow through their extremities to maintain the integrity of the tissue without resorting to the "hunting response". This appears to be an example of the "continuous proportional control" of blood flow suggested by Meyer and Webster (1971). Whereas the "hunting response" is perhaps due to periodic interruption of sympathetic neural activity to the larger blood vessels of an extremity. The "continuous proportional control" may represent either a more finely tuned version of the same mechanism (perhaps with the control vested in much smaller blood vessels such as the arterioles) or might involve a local build up of a dilator substance which counter balances the sympathetic neural constrictor activity. The first alternative is supported by the observation of a large negative arterio-venous difference in plasma noradrenaline concentration across the ear, only at times when the ear temperature was declining. There was little or no difference in noradrenaline concentration when the ear was constricted but maintaining a constant temperature.

The mechanisms by which sympathetic neural activity to an individual extremity might fluctuate selectively and independently to result in the "hunting response" or "continuous proportional control" have not been identified. There are several factors including histamine, dopamine, seratonin, noradrenaline, PG, and acetylcholine that can modulate release of noradrenaline locally in tissues (Westfall 1977). These substances might be likely candidates for achieving "continuous proportional control" either through modulation of noradrenaline release or possibly by direct dilatory effects on small arterioles. The coordination required to dilate and constrict an entire extremity as in the "hunting response" might be provided by a somatosympathetic reflex, similar to that described for the inhibition of cutaneous vasoconstrictor neurons in the hind foot of the cat (Janig 1975). It is conceivable that maximal vasoconstriction and cooling of an ear to near freezing temperatures provides a noxious stimulus which reflexively inhibits the ipsilateral but not the contralateral vasoconstrictor nerves to the ear vessels. Such a reflex could result in vasodilatation (i.e "hunting response") of one ear independently of the opposite ear and other extremities.

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APPENDIX I

Appendix Table 1. Design of preliminary trials in which compound 48/80 was infused into the right carotid artery of cold exposed sheep[1].

Trial	Animal[2]	Room temperature (C)	Dose mg/kg	Volume of infusate (ml)	Time after previous infusion (min)	Length of infusion (min)	Length of time monitored after each infusion (min)
1	0581	-18	1.0	20		2	45
		-21	1.0	20	100	23	45
2	1650[3]	-20	1.0	20		23	23
3	1674	-20	0.1	20		23	23
		-20	0.1	20	40	24	24
		-20	0.2	20	90	23	23
4	1647	-17	0.2	20		22	22
		-18	0.2	20	80	23	23
		-17	saline	20	47	23	23
		-17	0.2	20	18	24	24
		-16	0.3	20	26	21	21

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[1] Compound 48/80, lot. number 746-090, Sigma Chemical Company, St. Louis, Missouri, are described in Materials & Methods section of experiment 2 of chapter 2.

[2] 0581 and 1647 are Cheviot wethers weighing 60 and 90 kg respectively; 1650 and 1674 are Suffolk wethers weighing 85 and 89 kg respectively.

[3] Animal died 15 min after completion of monitored time showing symptoms similar to anaphylactic shock as described by Alexander et al. 1970.

Appendix Table 2. The effects of carotid artery infusion of compound 48/80 on maximum and minimum ear skin temperature, the mean of the maximum minus minimum ear skin temperature, the interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 1, Animal No. 0581

				After 48/80 Infusion (1 mg/kg)				
		Before Infusion		First Infusion		Second Infusion		
		Right ear	Left ear	Right ear	Left ear	Right ear	Left ear	SE[1]
Max. ear skin temp.	(C)	$12.0^{b,2}$	11.4^{b}	17.4^{a}	10.2^{b}	16.6^{a}	10.2^{b}	1.05
Min. ear skin temp.	(C)	2.9	3.9	3.9	2.7	3.2	1.9	0.37
Difference	(C)	8.9^{b}	7.5^{b}	13.8^{a}	7.5^{b}	13.5^{a}	8.2^{b}	1.27
Interval	(s)	301	531	404	450	397	430	33
Heart rate (beat/min)		172^{a}		236^{b}		224^{b}		17

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[1] SE = standard error of the mean.

[2] The harmonic mean of the number of observations per treatment was 7.32 for the interval and 8.93 for the other parameters.

[a,b,c] Means with different superscript letters within rows are significantly different ($P<0.05$).

Appendix Table 3. The effects of carotid artery infusion of compound 48/80 on maximum and minimum ear skin temperature, the maximum minus minimum ear skin temperature, the interval between "hunting responses" and heart rate in cold exposed sheep. Trial 2, Animal No. 1650.

		Before		After 48/80 infusion (1 mg/kg)		
		Right ear	Left ear	Right ear	Left ear	SE[1]
Max ear skin temp.	(C)	$14.7^{b,2}$	3.5^{c}	18.0^{a}	4.5^{c}	0.86
Min. ear skin temp.	(C)	2.7	0.5	8.4	1.0	0.35
Difference	(C)	12.0^{a}	3.0^{b}	9.6^{a}	3.5^{b}	0.80
Interval	(s)	359	321	355	204	50
Heart rate (beat/min)		192^{a}		232^{b}		14.8

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[1] SE = standard error of the mean.

[2] The harmonic mean of the number of observations per treatment was 3.21 for the interval and 5.45 for the other parameters.

a,b,c, Means with different superscripts within rows are significantly different ($P<0.05$; $P<0.25$ for heart rate).

Appendix Table 4. The effects of carotid artery infusion of compound 48/80 on maximum and minimum ear skin temperature, the mean maximum minus minimum ear skin temperature, the interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 3, Animal No. 1674.

		Before	After 48/80 infusion			
			(0.1 mg/kg)	(0.1 mg/kg)	(0.2 mg/kg)	SE[1]
Max ear skin temp.	(C)	13.4	15.8	14.0	12.2	0.66
Min. ear skin temp.	(C)	2.1	1.8	0.0	0.0	0.37
Difference	(C)	11.3	14.0	14.0	12.2	0.74
Interval	(s)	426	489	403	495	56
Heart rate (beat/min)		212	224	194	224	4

[1] SE = standard error of the mean.

[2] The harmonic mean of the number of observations per treatment was 4.31 for the interval and 5.39 for the other parameters.

Appendix Table 5. The effects of carotid artery infusion of compound 48/80 on maximum and minimum ear skin temperature, the mean of the maximum minus minimum ear skin temperature, the interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 4, Animal No. 1647.

	Before	After 48/80 Infusion				After Saline Infusion	SE[1]
		(0.2 mg/kg)	(0.2 mg/kg)	(0.2 mg/kg)	(0.3 mg/kg)		
Max. ear skin temp. (C)	8.0[2]	8.2	6.0	4.5	5.0	4.7	0.5
Min. ear skin temp. (C)	0.6	0.1	1.5	0.2	0.5	0.9	0.3
Difference (C)	7.4	8.1	4.3	4.7	4.3	5.2	0.6
Interval (s)	341	518	462	387	405	570	62.5
Heart rate (beat/min)	152	188^b	184^b	196^b	196^b	156^a	6

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[1] SE=standard error of the mean.

[2] The harmonic mean of the number of observations per treatment was 3.61 for the interval and 4.71 for the other parameters.

[a,b] -Means with different superscripts within rows are significantly different ($P<0.05$).

Appendix Table 6. Design of preliminary trials in which histamine-HCl was infused via the right carotid artery of cold exposed sheep.[1]

Trial	Animal[2]	Room temperature (C)	Dose µg/kg	Volume of infusate (ml)	Time after previous infusion (min)	Length of infusion (min)	Length of time monitored after each infusion (min)
1	1674	-21	0.50	10		20	17
		-20.5	0.25	15	18	17	20
		-21	0.50	10	40	30	30
		-21	0.50	10	50	2	18
		-21	0.50	10	18	2	45
2	1651[3]	-20	0.50	10		2	25
		-20	1.00	35	36	35	35
		-19.5	1.00	35	60	35	35
		-20	1.00	10	40	2	20

[1] All chemicals and procedures described for Experiment 2-2 in chapter 2.

[2] 1674 and 1651 Suffolk wethers weighing 89 and 80 kg respectively.

[3] Animal collapsed and died 130 minutes after completion of the fourth infusion.

Appendix Table 7. The effect of infusion of histamine-HCl via the right carotid artery on maximum and minimum ear skin temperatures, the mean of the maximum minus minimum ear skin temperature, the interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 1, Animal No. 1674.

Histamine dose (µg/kg)	Ear Skin Temperature (C)						"Hunting Response" Interval (s)		Heart Rate (beat/min)		
	Maximum		Minimum		Difference						
	Right ear	Left ear	Right ear	Left ear	Right ear	Left ear	Right ear	Left ear	Before[1]	After[1]	Increase
Preinfusion	8.1[2]	5.2	0.7	1.0	9.8[b]	5.6[b]	388	262			
0.50	5.7	4.6	0.4	0.7	5.9[b]	3.4[b]	353	203	138	150	12
0.25	7.7	4.8	-0.4	-0.1	8.6[b]	4.5[b]	334	208	150[a]	198[b]	48
0.50	9.3	4.7	-0.3	0.1	10.0[b]	4.2[b]	375	271	138	144	6
0.50	7.7	3.9	-1.2	0.6	9.4[b]	2.8[a]	223	187	120[a]	162[b]	42
0.50	8.2	6.3	- .03	-1.2	8.6[b]	6.9[b]	471	259	130[a]	192[b]	62
SE[3]	1.2		0.6		1.5		82				

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[1] Means were calculated for 3 min before and 15 to 45 min after infusion of histamine-HCl.

[2] The harmonic mean of the number of observations per treatment was 4.82 for the interval and 6.14 for the other parameters.

[3] SE = standard error of the mean.

[a,b]-Means with different superscripts differ significantly ($P<0.05$).

Appendix Table 8. The effect of infusion of histamine-HCl via the right carotid artery on maximum and minimum ear skin temperatures, the mean of the maximum minus minimum ear skin temperature, the interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 2, Animal No. 1651.

	Ear Skin Temperature (C)						"Hunting Response" Interval (s)		Heart Rate (beat/min)		
	Maximum		Minimum		Difference						
Histamine dose (µg/kg)	Right ear	Left ear	Right ear	Left ear	Right ear	Left ear	Right ear	Left ear	Before^{1}	After^{1}	Increase
Preinfusion	10.3^{2}	8.5	-0.2^{c}	0.0	10.8	9.1	344	371			
0.5	10.6	8.8	0.3^{abc}	0.5^{abc}	10.1	8.4	259	285	140^{a}	190^{b}	50
1.0	12.4	10.9	0.7^{abc}	0.8^{abc}	11.6	10.0	470	626	170^{a}	200^{b}	30
1.0	10.0	7.9	0.5^{abc}	0.8^{abc}	9.5	7.7	395	292	160^{a}	190^{b}	30
1.0	11.9	10.2	2.2^{ab}	2.4^{a}	9.6	7.9	457	519	140^{a}	240^{b}	100
SE^{3}	1.1		0.5		1.15		44.6				

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[1] Means were calculated for 3 min before and 15 to 45 min after histamine infusion.

[2] The harmonic mean of the number of observations per treatment was 6.76 for the interval and 7.72 for the other parameters.

[3] SE=standard error of the mean.

^{a,b,c}-Means with different superscripts differ significantly ($P<0.05$; $P<0.025$ for heart rate).

Appendix Table 9. Design of preliminary trials in which histamine-HCl infused via the jugular vein in cold exposed sheep.[1]

Trial	Animal[2]	Room Temperature (C)	Dose µg/kg	Volume of infusate (ml)	Time after previous infusion (min)	Length of infusion (min)	Length of time monitored after each infusion (min)
1	1649	-25	0.5	10		2	25
		-30	0.5	10	32	2	25
		-27	0.75	10	27	2	20
		-27	0.75	10	58	2	15
		-27	0.75	10	17	2	25
2	1691	-23	0.01	1.5		2	20
		-24	0.02	3.0	35	2	25
		-25	0.03	4.5	32	2	32
		-25.5	0.04	6.0	34	2	32
		-26.5	0.05	7.5	34	2	55
		-27.5	0.06	9.0	57	2	40
		-28.5	0.07	10.5	45	2	35
		-28.5	0.50	1.0	36	2	15
		-29.0	saline	9.0	23	2	15
		-29.0	0.66	0.6	23	2	16
		-29.5	1.0	2.0	28	2	20
3	0521	-24	0.01	1.3		2	25
		-25	0.02	2.7	28	2	20
		-26	0.03	3.9	27	2	30
		-28	0.04	5.2	60	2	10
4	0597	-21.5	0.01	1.3		2	20
		-22.0	0.01	1.3	83	2	25
		-23.5	0.02	2.7	28	2	25
		-22.5	0.4	.50	36	2	10
		-24	0.5	.65	15	2	20
		-24	0.5	.65	20	2	10
		-24	0.2	.24	10	2	10

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[1] All chemicals and procedures are identical to those described for experiment 2-2, chapter 2.

[2] 1649, Suffolk wether 79kg; 1691 and 0521, Suffolk x Cheviot wethers 30 and 65 kg, respectively; and 0597 Cheviot wether 65 kg.

Appendix Table 10. The effects of jugular infusion of histamine on the maximum and minimum ear skin temperatures, the mean of the maximum minus minimum ear skin temperature, interval between "hunting responses" and the heart rate in cold exposed sheep. Trial 1, Animal No. 1649.

Dose (μg/kg)	Ear Skin Temperature (C) Maximum	Minimum	Difference	"Hunting Response" interval (s)	Heart rate (beat/min)
Preinfusion	7.2[1]	2.6	2.6^{ab}	431	234
0.50	6.7	2.6	4.1^{b}	328	240
0.50	8.4	2.8	5.5^{ab}	335	240
0.75	8.5	1.8	6.7^{a}	650	240
0.75	5.6	1.6	3.9^{b}	116	230
0.75	5.6	1.3	4.3^{b}	331	220
SE[2]	0.31	0.16	0.62	65	2

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[1] The harmonic mean of the number of observations per treatment was 4.54 for the interval and 5.74 for the other parameters.

[2] SE-stantard error of the mean.

[a,b]-Means with different superscripts differ significantly ($P<0.05$).

Appendix Table 11. The effects of jugular infusion of histamine on the maximum and minimum ear skin temperatures, the mean of the maximum minus minimum ear skin temperature, interval between "hunting responses" and heart rate in cold exposed sheep. Trial 2, Animal No. 1691.

Dose (μg/kg)	Ear Skin Temperature (C)			"Hunting Response" interval (s)	Heart rate (beat/min)
	Maximum	Minimum	Difference		
Preinfusion	4.9^{c1}	-0.6^{c}	5.5	308	220
0.01	6.2^{bc}	0.4^{bc}	5.8	252	236
0.02	12.1^{a}	2.9^{abc}	9.2	415	224
0.03	9.2^{abc}	3.1^{abc}	6.0	319	216
0.04	8.2^{abc}	3.7^{ab}	4.4	246	224
0.05	10.6^{ab}	5.0^{a}	5.6	382	240
0.06	10.7^{ab}	4.5^{ab}	6.2	386	232
0.07	7.4^{bc}	3.6^{ab}	3.9	338	224
0.50	8.7^{abc}	3.3^{abc}	5.4	440	212
0.66	9.2^{abc}	4.2^{ab}	9.0	338	220
1.00	$-.2^{abc}$	2.3^{abc}	6.8	255	220
Saline	8.3^{abc}	0.9^{bc}	7.4	---	224
SE[2]	0.99	0.92	0.33	41	3

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[1] The harmonic mean of the number of observations per treatment was 2.11 for the interval and 3.74 for the other parameters.

[2] SE-standard error of the mean.

[a,b,c] -Means with different superscripts within columns differ significantly ($P<0.01$).

Appendix Table 12. The effects of jugular infusion of histamine on the maximum and minimum ear skin temperatures, the mean of the maximum minus minimum ear skin temperature, interval between "hunting responses" and heart rate in cold exposed sheep. Trial 3, Animal No. 0521.

Dose (μg/kg)	Ear Skin Temperature (C)			"Hunting Response" interval (s)	Heart rate (beat/min)
	Maximum	Minimum	Difference		
Preinfusion	8.4^{a1}	2.9^{a}	5.6	365	96
0.01	6.6^{ab}	2.0^{a}	4.7	234	132
0.02	6.5^{ab}	1.4^{ab}	5.1	259	132
0.03	5.2^{b}	-0.7^{c}	5.6	333	168
0.04	5.7^{b}	0.2^{bc}	5.5	285	156
SE[2]	0.59	0.52	0.36	35	11

[1] The harmonic means of the number of observations per treatment was 5.10 for the interval and 6.63 for the other parameters.

[2] SE-standard error of the mean.

[a,b,c] -Means with different superscripts within columns differ significantly ($P<0.05$).

Appendix Table 13. The effects of jugular infusions of histamine on the maximum and minimum ear skin temperatures, the neam of the maximum minus minimum ear skin temperature, interval between "hunting responses" and heart rate in cold exposed sheep. Trial 4, Animal No. Q597

Dose (µg/kg)	Ear Skin Temperature (C)			"Hunting Response" interval (s)	Heart rate (beat/min)
	Maximum	Minimum	Difference		
Preinfusion	2.9[1]	-1.5	4.3	590	110
0.01	2.0	-1.5	3.6	345	120
0.01	2.7	0.5	1.9	525	120
0.02	2.3	-0.5	2.8	360	120
0.2	0.8	-1.4	2.2	210	132
0.40	1.3	-1.5	2.8	---	168
0.50	1.7	-0.5	2.8	209	160
0.50	0.9	-1.1	2.1	150	170
SE[2]	0.29	0.28	0.28	82	9

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[1] The harmonic mean of the number of observations per treatment was 2.36 for the interval and 3.01 for the other parameters.

[2] SE-standard error of the mean.